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Effect of phosphorus level and phytase inclusion on the performance, bone mineral concentration, apparent nutrient digestibility, and on mineral and nitrogen utilisation in finisher pigs

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Two experiments were conducted to investigate the interaction between dietary P concentration and phytase (PHY) inclusion in the diet of finisher pigs. In Experiment 1, the growth performance and bone analysis experiment, pigs (6 replicate groups of 14 pigs each per treatment; initial body weight (BW) = 45.2 kg) were allocated to one of six dietary treatments (for 74 days) in a 3 × 2 factorial arrangement: T1 – available P in the diet = 1.5 g/kg; T2 = T1 with 500 units of phytase (FTU)/kg; T3 – available P = 2.0 g/kg; T4 = T3 with 500 FTU/kg; T5 – available P = 2.5 g/kg; T6 = T5 with 500 FTU/kg. Experiment 2 consisted of a digestibility and a P, Ca and N balance study, and pigs (6 per treatment; initial BW = 67.3 kg) were offered identical diets to those offered in Experiment 1. There was an interaction between dietary P level and PHY inclusion for average daily gain (ADG) and carcass weight (CW; $P < 0.05$) in Experiment 1. Pigs offered the low P diet supplemented with PHY had a higher ADG and CW than pigs offered the non-PHY, low P diet. However, there was no effect ($P > 0.05$) of PHY inclusion on ADG or CW with the medium or high P diets. Higher concentrations of ash, P and Ca in bone were noted in pigs offered the medium and high P diets ($P < 0.001$) and PHY ($P < 0.01$) diets when compared to pigs offered the low P without PHY. Pigs offered diets supplemented with PHY had lower faecal P output ($P < 0.01$) and a higher P digestibility ($P < 0.001$) and P retention ($P < 0.05$) than pigs offered diets without added PHY. In conclusion, supplementation of a low-P finisher diet with PHY resulted in pigs that had a similar carcass weight, but weaker bones than pigs offered a medium or high P diet.

Keywords: bone; digestibility; phosphorus; phytase; pig

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Introduction

Swine manure contains a high level of P, the result of poor digestibility of phytate P and the consumption of P above the pigs' requirements (Knowlton *et al.*, 2004). Environmental regulations limit the level of P to be discharged in swine effluent (Vats, Bhattacharyya and Banerjee, 2005; Selle and Ravindran, 2008). Hence, there is a need to reduce the dietary P level as well as to develop nutritional strategies to improve its bioavailability. Supplementing pig diets with phytase (PHY) is becoming increasingly common (Selle and Ravindran, 2008) as a method of improving the availability of P in plant ingredients containing high levels of phytate P (Brady *et al.*, 2003; Varley *et al.*, 2010).

Various P requirements during the finisher period can be derived, depending on the objective; pigs require less dietary P for optimal body growth than for optimal bone quality (NRC, 1998). The concentration of P in the diet for maximising bone ash concentration is at least 1 g/kg higher than the concentration required for maximum efficiency of gain (Cromwell *et al.*, 1970). However, environmental considerations demand that formulating diets to meet optimal bone mineralisation should only be used where mineralisation is required for either welfare or production reasons, or where the prior establishment of a bone reservoir is essential to meet ensuing peak demands (Underwood and Suttle, 2001). Although there is a great deal of interest in reducing P excretion by production animals through dietary manipulation of P, such efforts could result in subtle deficiencies of P that not only affect growth, but also adversely affect bone integrity. The prevention of dietary P deficiency is critical to maintaining the profitability of swine production as well as animal well-being. The need to minimise the amount of P accumulating in animal waste has introduced a new perspective into what constitutes a minimum requirement (Eeckhout *et al.*, 1995).

The objective of the present study was to investigate the interaction between the level of P in the diet and the inclusion of PHY for growth performance, bone mineralisation and total tract apparent digestibility, and P, Ca and N utilisation by finisher pigs. The hypotheses were that feeding the finisher pig different dietary P levels (available P levels of 1.5, 2.0 or 2.5 g/kg) would affect growth performance, mineral retention and bone mineral accretion; the inclusion of PHY (0 *vs.* 500 phytase units per kilogram) would eliminate the negative effects of low P inclusion on the aforementioned traits of finisher pigs.

Materials and Methods

All procedures described in this experiment were conducted under experimental licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act 1876) Regulations, 1994.

Experimental design and diets

Two experiments were designed as 3 × 2 factorials comprising six dietary treatments. The dietary treatments were as follows: T1 – available P = 1.5 g/kg; T2 = T1 with 500 units of phytase (FTU)/kg; T3 – available P = 2.0 g/kg; T4 = T3 with 500 FTU/kg; T5 – available P = 2.5 g/kg; T6 = T5 with 500 FTU/kg. The requirement for available P in the diet of a pig at 50 to 80 kg body weight (BW) is 1.9 g/kg (NRC, 1998). All diets were formulated to contain similar concentrations of net energy and ileal digestible lysine (INRA-AFZ, 2004). All other amino

acid requirements were met relative to lysine according to the ideal protein concept (NRC, 1998). The microbial PHY used was produced by *Aspergillus niger* and was added in granulated form (Natuphos 5,000 – BASF Corporation, Ludwigshafen, Germany). All diets were offered in meal form; the composition and analysis of diets are presented in Table 1.

Experiment 1

The pigs used (252 entire males and 252 females) were the progeny of Landrace × Large White sows and Meatline boars, initial BW was 45.2 (s.d. 2.5) kg and age at the start was approximately 13 weeks. The pigs were assigned to six blocks on the basis of initial BW and gender and were allocated at random to one of the

Table 1. Composition and chemical analysis of experimental diets (g/kg, as-fed)

	Diet: P concentration by phytase inclusion					
	Low		Medium		High	
	No	Yes	No	Yes	No	Yes
Ingredient (g/kg)						
Wheat	466.7	466.6	462.7	462.6	458.7	458.6
Barley	300.0	300.0	300.0	300.0	300.0	300.0
Soya bean meal	200.0	200.0	200.0	200.0	200.0	200.0
Soya bean oil	10.0	10.0	10.0	10.0	10.0	10.0
L-Lysine	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	0.8	0.8	0.8	0.8	0.8	0.8
L-Threonine	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0	0	4.0	4.0	8.0	8.0
Limestone	13.0	13.0	13.0	13.0	13.0	13.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Vitamins and minerals[1]	2.5	2.5	2.5	2.5	2.5	2.5
Phytase[2]	0	0.1	0	0.1	0	0.1
Chemical analysis[3] (g/kg)						
Dry matter	869.8	872.3	870.7	871.9	863.5	866.5
Crude protein	173.9	175.2	174.6	175.2	173.8	175.3
Neutral detergent fibre	131.7	132.5	132.0	131.9	131.6	132.1
Ash	42.6	40.4	44.3	45.7	46.0	48.0
Gross energy (MJ/kg)	16.0	15.9	15.9	15.8	15.8	15.9
Phosphorus	3.7	3.7	4.4	4.4	5.3	5.2
Calcium	5.9	5.9	7.0	7.1	8.1	8.2
Lysine	9.6	9.4	9.3	9.5	9.6	9.5
Methionine and cystine	5.8	5.7	5.6	5.8	5.6	5.7
Threonine	5.9	5.9	5.8	5.7	5.9	5.8
Phytase activity (FTU/kg)	101	601	94	597	89	622
Calculated composition[4]						
Available phosphorus (g/kg)	1.5	1.5	2.0	2.0	2.5	2.5
Net energy (MJ/kg)	9.8	9.8	9.8	9.8	9.8	9.8

[1] Premix provided (mg/kg of complete diet): retinol 3, cholecalciferol 0.05, alpha-tocopherol 40, copper 25 (as copper II sulphate), zinc 100 (as zinc oxide), selenium 0.03 (as sodium selenite), manganese 25 (as manganous oxide) and iodine 0.2 (as calcium iodate on a calcium sulphate/calcium carbonate carrier).
[2] Natuphos 5,000 – BASF Corporation, Ludwigshafen, Germany.
[3] Four replicate analyses.
[4] INRA-AFZ (2004).

six dietary treatments. The pigs were penned in mixed groups of 14 (7 males and 7 females) and were stocked at 0.8 m^2 per pig, with 6 replicate pens per treatment. Prior to the experiment, pigs received standard commercial feeding (ration had available P of 3.5 g/kg and Ca of 7.0 g/kg) and management. The house was mechanically ventilated to provide an ambient temperature of 18 °C. Each pen had a solid-floor lying area with access to slats at the rear. Individual single space feeders with water nipples were present in all pens providing an *ad libitum* supply of both food and water. Feed intake was measured by recording the feed disappearance from the feeder. Animals were individually tagged and were weighed on days 0 (start of experiment), 28 and 74.

All the pigs were removed for slaughter on day 74. After overnight fasting (for ~15 h) animals were transported to a commercial slaughter plant, rested for 2 h, stunned by CO_2 and killed by exsanguination. Measurements of backfat and muscle depths were taken on the carcass at a point 6 cm from the edge of the split back at the level of the 13^{th} and 14^{th} rib using a Hennessy Grading Probe (HGP, Hennessy and Chong, Auckland, New Zealand).

The lean meat in the carcass (g/kg) was estimated (Department of Agriculture and Food, 1994) as $543.1 - 7.86x + 2.66y$; where x = backfat depth (mm) and y = muscle depth (mm). Carcass weight (CW) was calculated as hot carcass weight × 0.98 and kill-out proportion was calculated as CW divided by final BW.

Immediately after slaughter, the two front feet (2 pigs per pen; 1 male and 1 female who were randomly chosen; n=6) were severed at the knee joint from each leg. The collected feet from each individual pig were placed in a plastic bag and were stored at –20 °C until required for laboratory analysis.

Experiment 2

Thirty-six finishing boars (initial BW 67.3 (s.d. 2.1) kg) were used to estimate the coefficients of total tract apparent digestibility, and P, Ca and N utilisation. This experiment was carried out in conjunction with the performance study (Experiment 1) using pigs of the same genetic stock and previous history. The boars were blocked on the basis of BW and were randomly assigned to the six treatments used in Experiment 1. The boars were allowed a 21-day dietary adaptation period and then weighed and transferred to individual metabolism crates that facilitated total, but separate, collection of urine and faeces. The pigs were given 6 days to acclimatise to the metabolism crates followed by a 7-day collection period. The daily feed allowance offered was calculated from the required digestible energy intake of $3.44 \times BW^{0.54}$ MJ/day (Close, 1994), and was divided over two meals. Water was provided with the feed in a 1:1 ratio. Between meals, fresh water was provided *ad libitum*. The metabolism crates were located in a temperature controlled room maintained at 22 (±1.5) °C. During the collection period urine was collected in a plastic container, via a funnel below the crate. To avoid N volatilisation, 20 mL of 4.5 M sulphuric acid was added to the container daily. Urine output was recorded daily and a 50 mL sample was retained (frozen) for laboratory analysis. Total faecal collection was performed each morning and weighed; an aliquot (100 g) was retained and frozen (–20 °C) for subsequent N determination and the remaining faeces were oven dried at 100 °C. Feed samples were collected each day, pooled, frozen (–20 °C) and retained for chemical analysis.

Chemical analysis

The analysis of diets and faeces for dry matter (DM), by method 934.01, and crude ash, by method 942.05, was carried out according to the Association of Official Analytical Chemists (1997). The DM was determined after drying samples for 24 h at 100 °C. The crude ash was determined after ignition of a weighed sample in a muffle furnace (Nabertherm, Bremen, Germany) at 500 °C for 6 h. The ash was then digested in aqua regia (80 mL 12 M hydrochloric acid/20 mL 16 M nitric acid). This solution was used for P and Ca determination. Calcium concentration was determined using a Varian '50' atomic absorption spectrophotometer (5301 Stevens Creek Boulevard, Santa Clara, CA 95051, USA) (Ramakrishna, West and Robinson, 1968). The concentration of P was determined spectrophotometrically (Pye-Unicam PU 8600 UV/visible spectrophotometer, Philips, UK) using the method of Cavell (1955). Phosphorus in the urine was determined using the method of Fiske and Subbarow (1925). The gross energy of diets and faeces samples was determined using an adiabatic bomb calorimeter (Parr Instruments, IL, USA). The neutral detergent fibre (NDF) fraction of diets and faeces was analysed using a Fibertec extraction unit (Tecator, Hoganans, Sweden) as described by Van Soest, Robertson and Lewis (1991). The N concentration of diets and urine was determined using a LECO FP 528 instrument (Leco Instruments, UK Ltd, Newby Road, Hazel Grove, Stockport, SK7 5DA, Cheshire, UK). The N concentration of fresh faeces was analysed by the macro-Kjeldahl technique using a Buchi digestion K-437 and distillation K-360 apparatus (Buchi Labortechnik, AG-CH-9230, Flawil, Switzerland). The dietary concentrations of lysine, threonine, methionine and cysteine were determined by high-performance liquid chromatography (Varian Prostar, Varian, Walnut Creek, CA, USA) as described by Iwaki *et al*. (1987). Feed samples were analysed for PHY activity according to the method reported by Brady *et al.* (2003) and expressed as FTU per kilogram of feed. One FTU is defined as the quantity of enzyme that liberates 1 μmol of inorganic P per minute from a 1.5 mmol/L solution of sodium phytate at pH 5.5 and 37 °C (Engelen *et al.*, 2001).

Bone analysis

The pooled individual pig feet samples were cleaned of all skin, muscle and connective tissue in order to remove the third and fourth metacarpal bones from the foot. Following removal, the metacarpals were again cleaned of any remaining flesh. Bones were analysed for ash, Ca and P concentrations as described by Brady *et al.* (2002). The bone was placed in an oven at 100 °C for 24 h to determine the DM weight. The sample was then placed in a muffle furnace at 650 °C for 4 h and the ash was digested in aqua regia (HCl/HNO_3 mixture). This solution was used for P and Ca determination as described above.

Statistical analysis

The data from Experiments 1 and 2 were analysed as 3 × 2 factorials using the Proc GLM (SAS, 1985). The statistical model for all analysis included terms for P, PHY, P × PHY and block. All data were checked for normality using the Proc UNIVARIATE (SAS, 1985). The Tukey-Kramer test was used for pairwise comparison of means. The bone data and carcass characteristics were adjusted using slaughter weight as a covariate. For finisher pig performance and bone composition, the pen was the experimental unit. The individual pig served as the experimental unit for data from Experiment 2. Significant

differences were based on a Type 1 error rate of 0.05.

Results

Experiment 1

The effects of dietary treatment on finisher pig performance, carcass characteristics and bone mineral concentration are presented in Table 2. Treatment had no effect on growth rate between days 0 and 28. Pigs offered the high P diet had a higher average daily feed intake (ADFI) between days 28 and 74 when compared to pigs offered the low and medium P diets (2.39 *vs.* 2.23 and 2.23 kg/day, s.e. 0.046; $P < 0.05$). Pigs offered the high and medium P diets had a higher ($P < 0.05$) ADG (0.862 and 0.832 *vs.* 0.766 kg/day, s.e. 0.022) and a better feed conversion ratio (FCR; 2.77 and 2.69 *vs.* 2.92, s.e. 0.057) over the period from day 28 to 74 compared to pigs offered the low P diets. Pigs offered diets with added PHY had higher ($P < 0.05$) ADG (0.845 *vs.* 0.795 kg/day, s.e. 0.018) and better FCR (2.73 *vs.* 2.86, s.e. 0.046) between days 28 and 74 than pigs offered non-PHY diets. Pigs offered the high P diets had a higher ADFI overall (days 0 to 74) than pigs offered the low or medium P diets (2.16 *vs.* 2.04 and 2.04 kg/day, s.e. 0.033; $P < 0.05$). Pigs offered the high and medium P diets had an improved FCR overall compared to pigs offered the low P diets (2.57 and 2.54 *vs.* 2.70, s.e. 0.037; $P < 0.05$). There was an interaction between P level in the diet and PHY inclusion for ADG from 0 to 74 days, and for final BW and CW ($P < 0.05$). Pigs offered the low P diet supplemented with PHY had a higher ADG, higher final BW and higher CW than pigs offered the low P diet without PHY. However, there was no effect of PHY inclusion in the medium or high P diets for ADG, final BW or CW. The bones from pigs offered the medium and high P diets had a higher ($P < 0.001$) concentration of ash (504.6 and 516.9 *vs.* 457.6 g/kg, s.e. 9.76), P (85.8 and 87.9 *vs.* 77.8 g/kg, s.e. 1.66) and Ca (181.7 and 186.1 *vs.* 164.7 g/kg, s.e. 3.51) compared to pigs offered the low P diets. Pigs offered diets with added PHY had a higher ($P < 0.01$) concentration of ash (512.9 *vs.* 473.3 g/kg, s.e. ± 7.97), P (87.2 *vs.* 80.5 g/kg, s.e. 1.36) and Ca (184.6 *vs.* 170.4 g/kg, s.e. 2.869) in bone than pigs offered diets without PHY.

Experiment 2

The effects of dietary treatment on nutrient digestibility and on the utilisation of N, Ca and P are presented in Table 3. Pigs offered diets with added PHY had a higher NDF digestibility compared to pigs offered non-PHY diets (528 *vs.* 461 g/kg, s.e. 21; $P < 0.05$). Pigs offered diets with added PHY had a lower ($P < 0.05$) urinary N output (21.9 *vs.* 24.6 g/day, s.e. 0.93) and total N excretion (30.3 *vs.* 33.6 g/day, s.e. 1.004) than pigs offered diets without PHY. Pigs offered diets with added PHY had higher ($P < 0.05$) N retention (32.2 *vs.* 29.4 g/day, s.e. 0.97) and N absorption coefficient (0.51 *vs.* 0.46, s.e. 0.016) than pigs offered diets without PHY. As dietary P level increased, P intake (7.3 *vs.* 8.9 *vs.* 10.1 g/day, s.e. 0.145; $P < 0.001$), output of P in faeces (3.4 *vs.* 4.0 *vs.* 4.6 g/day, s.e. 0.17; $P < 0.001$) and total P output (3.5 *vs.* 4.2 *vs.* 5.0 g/day, s.e. 0.19; $P < 0.001$) increased. Pigs offered high P diets had higher urinary output of P than pigs offered low P diets (0.35 *vs.* 0.07 g/day, s.e. 0.069; $P < 0.05$), whereas the output of P in urine by pigs on the medium P diets was not statistically different from either. Pigs offered the medium and high P diets had higher P retention compared to pigs offered the low P diet (4.7 and 5.1 *vs.* 3.8 g/day, s.e. 0.20; $P < 0.001$). Pigs offered diets with added PHY had lower P output in faeces (3.7 *vs.* 4.3 g/day, s.e. 0.15; $P < 0.01$) and

Table 2. Least squares means for effects of dietary P level and phytase inclusion on pig performance, carcass traits and bone mineral concentration: Experiment 1

Variable	Treatment[1]: P concentration by phytase inclusion							Significance test for		
	Low		Medium		High			P	PHY	P × PHY
	No	Yes	No	Yes	No	Yes	s.e.			
Initial body weight (kg)	45.2	45.3	44.9	45.4	45.2	45.1	2.06			
Final body weight (kg)	98.2[a]	104.6[b]	103.2[b]	106.2[b]	108.4[b]	106.4[b]	1.67	**		*
Day 0 to 28										
Average daily feed intake (kg/day)	1.72	1.74	1.71	1.76	1.82	1.75	0.031			
Average daily gain (kg/day)	0.732	0.746	0.759	0.763	0.820	0.760	0.0211			
Feed conversion ratio (kg/kg)	2.36	2.33	2.25	2.31	2.22	2.30	0.059			
Day 28 to 74										
Average daily feed intake (kg/day)	2.15	2.31	2.21	2.26	2.43	2.35	0.065	*		
Average daily gain (kg/day)	0.707	0.826	0.813	0.850	0.865	0.860	0.0312	*	*	
Feed conversion ratio (kg/kg)	3.05	2.80	2.71	2.66	2.81	2.73	0.080	*	*	
Day 0 to 74										
Average daily feed intake (kg/day)	1.99	2.09	2.02	2.07	2.20	2.12	0.046	*		
Average daily gain (kg/day)	0.716[a]	0.800[b]	0.788[b]	0.821[b]	0.853[b]	0.827[b]	0.0212	*		*
Feed conversion ratio (kg/kg)	2.77	2.62	2.56	2.52	2.58	2.56	0.053	*		
Carcass characteristics										
Carcass weight (kg)	75.1[a]	80.3[b]	79.0[b]	81.6[b]	83.8[b]	81.7[b]	1.32	**		*
Kill-out proportion (%)	76.4	76.7	76.5	77.0	77.3	76.8	0.45			
Fat depth (mm)	12.7	13.1	12.1	12.0	12.3	12.5	0.36			
Lean yield (%)	57.1	56.6	57.6	57.5	57.5	57.2	0.37			
Bone mineral concentration[2] (g/kg)										
Ash	439.3	475.8	479.8	529.5	500.6	533.3	13.73	***	**	
P	74.7	80.9	81.6	90.0	85.1	90.7	2.33	***	**	
Calcium	158.2	171.3	172.7	190.6	180.2	191.8	4.94	***	**	

[1] Six replicates (14 pigs/replicate) per treatment.
[2] Six replicates (2 pigs/replicate) per treatment.
[a,b] Means with different superscripts are significantly different ($P < 0.05$).

Table 3. Least squares means for effects of P concentration in the diet and phytase inclusion on total tract apparent digestibility and on the utilisation of N, P and Ca: Experiment 2

	Treatment[1]: P concentration by phytase inclusion						s.e.	Significance[2] test for	
	Low		Medium		High				
	No	Yes	No	Yes	No	Yes		P	PHY
Dry matter intake (kg/day)	1.85	1.86	1.83	1.86	1.90	1.85	0.033		
Digestibility (g/kg)									
Dry matter	875	885	866	875	880	872	6.1		
Ash	634	639	602	628	615	589	19.0		
Organic matter	888	897	880	888	895	888	6.1		
Neutral detergent fibre	475	536	406	531	503	516	36.0		*
Gross energy	869	876	860	868	878	870	7.0		
N balance									
N intake (g/day)	62.96	62.73	61.65	61.96	64.35	62.87	1.187		
Faecal N output (g/day)	9.32	8.17	10.00	8.57	7.70	8.43	0.742		
Urinary N output (g/day)	26.28	23.60	22.55	20.26	24.99	21.86	1.640		*
Total N excreted (g/day)	35.59	31.78	32.55	28.83	32.69	30.29	1.777		*
N retained (g/day)	27.36	30.95	29.10	33.13	31.66	32.58	1.715		*
N digestibility (g/kg)	853	869	842	861	881	866	12.0		
N absorption coefficient	0.44	0.50	0.47	0.53	0.50	0.51	0.028		*
P balance									
P intake (g/day)	7.30	7.31	8.95	8.80	10.01	10.18	0.204	***	
Faecal P output (g/day)	3.84	3.03	4.37	3.62	4.78	4.50	0.243	***	**
Urinary P output (g/day)	0.07	0.07	0.14	0.30	0.23	0.48	0.098	*	
Total P excreted (g/day)	3.92	3.10	4.50	3.91	5.02	4.98	0.277	***	*
P retained (g/day)	3.38	4.21	4.45	4.89	4.99	5.20	0.282	***	*
P digestibility (g/kg)	476	584	507	586	522	556	25.0		***
P absorption coefficient	0.46	0.57	0.49	0.55	0.50	0.51	0.029		*
Ca balance									
Ca intake (g/day)	10.77	11.69	13.43	13.56	15.05	14.47	0.554	***	
Faecal Ca (g/day)	5.62	5.39	8.18	6.78	8.46	7.87	0.525	***	*
Ca retained (g/day)	5.14	6.29	5.26	6.78	6.58	6.60	0.628		*
Ca digestibility (g/kg)	462	541	385	496	364	456	41.0		**

[1] Six pigs per treatment combination.
[2] There was no interaction between P level and PHY inclusion for the coefficients of total tract apparent digestibility, or for utilisation of N, P or Ca.

total P output (4.0 *vs.* 4.5 g/day, s.e. 0.16; $P < 0.05$), higher P digestibility (575 *vs.* 502 g/kg, s.e. 14; $P < 0.001$) and P retention (4.8 *vs.* 4.3 g/day, s.e. 0.16; $P < 0.05$) than pigs offered diets without PHY. As the level of P in the diet increased, Ca intake increased (11.2 *vs.* 13.5 *vs.* 14.8 g/day, s.e. 0.39; $P < 0.001$) as did output of Ca in faeces (5.5 *vs.* 7.5 *vs.* 8.7 g/day, s.e. 0.37; $P < 0.001$). Pigs offered diets with added PHY had lower output of Ca in faeces (6.7 *vs.* 7.7 g/day, s.e. 0.29; $P < 0.05$) and a higher Ca digestibility (498 *vs.* 404 g/kg, s.e. 23; $P < 0.01$) than pigs offered diets without PHY.

Discussion

The hypotheses tested in this experiment were that reducing the P level in the diet of finisher pigs would affect

growth performance, mineral retention and bone mineral accretion and that the inclusion of phytase would eliminate the negative effects of low P inclusion. The observed interaction between the level of P in the diet and PHY inclusion for growth performance (Experiment 1), and the main effects of P and PHY on bone mineralisation (Experiment 1) and P and Ca utilisation (Experiment 2) support these hypotheses.

The improvement in ADG and FCR as dietary P level increased (Experiment 1) is in line with results obtained from the mineral utilisation study (Experiment 2), where pigs offered the medium or high P diets had a proportional improvement in P retention of 0.22 compared to pigs offered the low P diet. The improvement in ADG and FCR with the inclusion of PHY is attributed to the ability of PHY to hydrolyse the phytate molecule (Selle and Ravindran, 2008) releasing bound P, Ca and N which was demonstrated in Experiment 2. Furthermore, there was an interaction between dietary P level and PHY inclusion for ADG overall, final BW and CW. The inclusion of PHY in the low P diet yielded an improvement in ADG, final BW and CW. However, there was no effect of PHY inclusion in the medium or high P diets for these variables. The requirement for available P in the diet for pigs weighing 50 to 80 kg is 1.9 g/kg (NRC, 1998). However, the low P diet in the current study had an available P level of only 1.5 g/kg, which is inadequate. Thus adding PHY to finisher-pig diets containing inadequate P can increase growth rate and produce pigs with a similar CW to pigs offered the recommended (NRC, 1998) concentration of dietary P. In agreement with the present results, O'Quinn, Knabe and Gregg (1997) demonstrated that supplementing the diet of finisher pigs with PHY at 300 FTU/kg, when the concentration of total P was 3.8 g/kg, yielded an increase in growth rate similar to that of finisher pigs offered a diet with a total P concentration of 4.3 g/kg. In addition, pigs offered the high P diet in the present study, had a higher ADFI from day 28 to 74 and from day 0 to 74 than pigs offered low and medium P diets; however daily feed consumption did not differ between treatments in the digestibility study. This may be due to a housing effect as the pigs in the metabolism crates may not exhibit the same appetite potential as pigs housed in groups in the growth performance study.

In the current study the inclusion of PHY yielded an increase in the digestibility of P, Ca and NDF, and in N absorption resulting in greater P, Ca and N retention compared to pigs offered diets without PHY. The capacity of microbial PHY to improve total tract apparent digestibility of P and Ca is well established (Selle and Ravindran, 2008), whereas the ability of PHY to increase the utilisation of NDF and N is less consistent. The observed increase in the digestibility of NDF may be due the ability of PHY to improve the accessibility of microbial enzymes to dietary fibre and thereby improve NDF fermentation (O'Doherty *et al*., 2010). The basis for the protein response to added PHY remains largely speculative. It may be that PHY prevents the formation of protein-phytate complexes within the gut, rendering the protein more susceptible to breakdown by pepsin in the stomach (Kies *et al.*, 2006). Alternatively, hydrolysis of the phytate molecule, via the inclusion of the PHY enzyme, releases phytate-bound protein (Selle and Ravindran, 2008). In agreement with the present results, Johnston *et al.* (2004) observed proportional increases of 0.10, 0.08 and 0.12 in the digestibility of P, Ca and NDF, respectively, when growing pigs were offered a diet containing an available P concentration of 1.9 g/kg

plus PHY level of 500 FTU/kg. Brady *et al.* (2003) demonstrated a proportional improvement of 0.03 in N digestibility with the inclusion of PHY (500 FTU/kg) in a finisher pig diet with a total P concentration of 4.4 g/kg.

In Experiment 1, pigs offered the medium or high P diets or diets supplemented with PHY had greater concentrations of ash, P and Ca in bone than pigs offered the low P diets or diets without PHY. The observed improvements may be attributed to the increase in P and Ca intake as dietary P and Ca concentration increased, and also to the increase in the digestibility and retention of Ca and P due to supplementation with PHY. Bone criteria are more sensitive and reliable indicators of P bioavailability than growth performance (Koch and Mahan, 1985). Because there was no difference between the medium and high P diets for bone mineral accretion, offering high P diets to finisher pigs to maximise the concentrations of ash, Ca and P in bone is not warranted. Furthermore the inclusion of PHY yielded a response in bone mineral concentration at all three dietary P levels, demonstrating that storage of Ca and P in bone continues even after the dietary needs for other functions have been met (Mahan, 1982). The results demonstrate that pigs require less dietary P for optimal growth than for optimal bone quality.

The need to minimise the amount of P in animal waste has introduced a new perspective on what constitutes a minimum requirement to meet the pigs' needs without comprising growth performance or bone development (Eeckhout *et al.*, 1995). The results from this study show that there is no advantage from offering finisher pigs dietary levels of available P > 2.0 g/kg in order to enhance growth performance or the concentrations of ash, Ca and P in bone. However, as dietary P level increased from low P to high P with PHY supplemented diets, there was a proportionate increase of 0.60 in total P excretion. The observed increase in urinary P excretion by pigs offered high P diets in Experiment 2 can be attributed to available P intake in excess of requirements, whereas faecal P excretion is attributed to inherently poor P availability (Selle and Ravindran, 2008). Since environmental regulations have set strict limits on the level of P to be discharged in swine effluents, dietary manipulation, by reducing dietary P below NRC (1998) requirements and including PHY would be an environmental benefit without adversely affecting growth performance. However there may be implications for breeding animals that are not selected from the herd until the latter part of the finishing period when bone mineralisation is largely completed (Varley *et al.*, 2011).

Conclusion

The supplementation of phytase in finisher pig diets containing 1.5 g aP/kg (45 to 100 kg BW), which is below NRC (1998) recommendations, produced pigs of a similar final BW and CW when compared to pigs offered a diet containing 2.0 g aP/kg and 2.5 g aP/kg. There is no advantage to feeding finisher pigs P levels greater than NRC (1998) requirements (1.9 g aP/kg) on bone mineral concentration at 100 kg BW. Because there was no difference between the medium and high P diet for bone mineral accrual, feeding high P diets to finisher pigs so as to maximise bone ash, bone Ca and bone P levels are not warranted.

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