Table 2. Nutrient and chemical composition of experimental diets (as-fed basis; phase 2)

Item	Positive control	Negative control 1[z]	Negative control 2[y]
Ingredient (%)			
Corn	72.04	73.24	73.69
Soybean meal	23.10	22.95	22.90
Pork fat	1.70	1.25	1.05
Salt	0.40	0.40	0.40
L-Lys HCl	0.30	0.30	0.30
DL-Met	0.08	0.08	0.08
L-Thr	0.08	0.08	0.08
Dicalcium phosphate	0.90	0.40	0.20
Limestone	0.90	0.80	0.80
Vitamin-mineral premix[x]	0.50	0.50	0.50
Calculated analysis			
AME (kcal kg^{-1})	3,100	3,100	3,100
CP (%)	16.50	16.50	17.00
Digestible lysine (%)	0.96	0.96	0.96
Ca (%)	0.61	0.50	0.45
Total P (%)	0.54	0.44	0.39
Available P (%)	0.27	0.17	0.12
Analysed composition			
DM (%)	87.10	87.80	87.80
Ether extract (%)	4.27	3.56	3.34
CP (%)	16.00	16.70	17.60
Ca (%)	0.61	0.57	0.36
Total P (%)	0.59	0.46	0.41

[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. 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.

Data were analysed using the least square means procedure in Minitab (v. 14–13th edition, Minitab Ltd. 2004, Coventry, UK). Pen served as the experimental unit for performance and pig was the experiment until for bone breaking strength and metacarpal ash. The model included block and treatment. Significance was accepted at $P<0.05$. Significant means were separated using Tukey's Highly Significant Difference test.

RESULTS AND DISCUSSION

The analysed dietary CP, ether extract, Ca and total P are presented in Tables 1 and 2 and confirm target levels. Phytase activities recovered in the diets were similar to formulated values when assay and sampling variation are considered (Table 3). The NC diets were formulated with reductions in Ca and avP. However, overall (day 0 to day 43) ADG or G:F were not different between pigs fed the PC, NC 1 or NC 2 diets and these results were not expected (Table 4). Average daily gain and G:F were significantly reduced in growing pigs fed diets formulated with 0.21% reductions in avP and only 0.12% reductions in Ca from 25 to 120 kg (Kuhn and Manner 2012). Body weight gain of 45 kg pigs was more influenced by dietary P levels rather than dietary Ca levels and the rate of gain increased as total P supplementation increased from 0.2 to 0.6%, regardless of the level of Ca in the diet (Chapman et al. 1962). More recently, there was no influence on growth performance of PIC337 or PIC280 growing pigs fed diets deficient in total P by 20% compared with pigs fed adequate P diets (Alexander et al. 2008). In the current trial, avP was reduced in the NC diets approximately 18 and 22% and thus this may not have been enough to elicit a significant depression in growth from day 0 to day 43.

Table 3. Recovered phytase activity of feed samples[zy]

Diet	Expected phytase activity (FTU kg^{-1})[x]	Recovered phytase activity (FTU kg^{-1})
Phase 1		
Positive control	0	<50
Negative control (NC) 1	0	<50
NC 1+250 (FTU kg^{-1})	250	260
NC 2	0	<50
NC 2+500 (FTU kg^{-1})	500	619
NC 2+2000 (FTU kg^{-1})	2,000	2,070
Phase 2		
Positive control	0	<50
NC 1	0	<50
NC 1+250 (FTU kg^{-1})	250	233
NC 2	0	<50
NC 2+500 (FTU kg^{-1})	500	446
NC 2+2,000 (FTU kg^{-1})	2,000	2,360

[z]Means represent the average of triplicate analyses per sample.
[y]Phytase recovered in the diets was analysed as described by Engelen et al. (2001).
[x]One phytase unit (FTU) is defined as the amount of enzyme required to release one µM of inorganic P per minute from sodium phytate at 37°C and pH 5.5.

Average daily gain in the younger growing pigs (23 to 37 kg) and bone breaking strength or bone ash weight in the 55-kg pigs appeared to be more susceptible to the low Ca and avP levels in the NC 2 diet than growth performance in the older pigs or bone ash percent. Low dietary Ca and P levels did not influence ADG in pigs at any age from 28 to 192 d post-weaning (Crenshaw et al. 1981) and the effect was negated in 37- to 55-kg pigs in the current trial. In addition, Crenshaw et al. (1981)

Table 4. Influence of diet on growth performance and metacarpal ash of pigs from 23 to 55 kg[z]

Item	Positive control[y]	NC 1[x]	NC 1+250 (FTU kg^{-1})	NC 2[w]	NC 2+500 (FTU kg^{-1})	NC 2+2000 (FTU kg^{-1})	SEM	*P* value
Body weight (kg)								
Initial (day 0)	22.8	22.9	22.9	22.9	22.9	22.9	0.15	0.98
Final (day 43)	55.1*c*	55.7*bc*	56.2*ab*	54.7*c*	56.7*ab*	57.3*a*	0.10	***
Phase 1 (day 0 to day 21)								
ADFI[v] (kg)	1.30	1.32	1.32	1.31	1.34	1.34	0.11	0.52
ADG[v] (kg)	0.68*b*	0.68*b*	0.68*b*	0.63*c*	0.72*a*	0.71*ab*	0.11	***
G:F[v] (kg kg^{-1})	0.527*ab*	0.518*b*	0.516*b*	0.479*c*	0.535*a*	0.532*ab*	0.06	***
Phase 2 (day 22 to day 43)								
ADFI (kg)	1.70	1.76	1.75	1.71	1.75	1.80	0.10	0.24
ADG (kg)	0.82*c*	0.83*bc*	0.87*ab*	0.85*abc*	0.85*abc*	0.89*a*	0.09	*
G:F (kg kg^{-1})	0.482*bc*	0.475*c*	0.498*a*	0.498*ab*	0.490*abc*	0.499*a*	0.05	*
Overall (day 0 to day 43)								
ADFI (kg)	1.50	1.55	1.54	1.51	1.55	1.57	0.10	0.22
ADG (kg)	0.75*c*	0.76*bc*	0.78*ab*	0.74*c*	0.78*ab*	0.80*a*	0.08	***
G:F (kg kg^{-1})	0.501*bc*	0.493*c*	0.506*ab*	0.490*c*	0.509*ab*	0.513*a*	0.04	***
Metacarpal measurements (55 kg)								
Breaking strength (kg)	77.4*abc*	69.5*bc*	80.9*ab*	65.9*c*	81.2*ab*	85.9*a*	0.06	*
Ash (%)	42.3	39.5	41.2	39.4	40.3	41.3	0.08	0.23
Ash weight (g)	3.64*a*	3.17*b*	3.57*a*	3.17*b*	3.61*a*	3.76*a*	0.03	***

[z]Data are means of eight replicates and six pigs per replicate pen.
[y]The positive control was formulated to be adequate in all nutrients.
[x]NC, negative control 1 was formulated with a reduction in Ca and available P from the PC by 0.11 and 0.10%, respectively.
[w]NC, negative control 2 was formulated with a reduction in Ca and available P from the PC by 0.16 and 0.15%, respectively.
[v]ADFI, average daily feed intake, ADG, average daily gain; G:F, gain to feed ratio.
a–c Means within rows with different letters are different ($P < 0.05$).
*, *** $P < 0.01$ and $P < 0.05$, respectively.

determined there was a non-significant relationship between the percentage of bone ash and bone strength parameters and any relationship, albeit poor, is dependent on the bone evaluated, the age of the pig, and the Ca and P level of the diet. Therefore, in the current trial, the lack of a significant effect of diet on metacarpal ash percentage may indicate bone ash percentage is not as sensitive a measure of mineral concentration as breaking strength or ash weight, particularly in the absence of growth responses to reduced dietary Ca and avP.

Phytase supplementation at 500 or 2000 FTU kg^{-1} in NC 2 improved ($P < 0.05$) ADG and G:F compared with pigs fed NC 2 from day 0 to day 21 and overall (day 0 to day 43). This has been previously reported in growing pigs fed avP and Ca deficient diets with 500 FTU kg^{-1} phytase (Kuhn and Manner 2012) or young pigs fed nutritionally adequate diets and 2500 FTU kg^{-1} phytase (Walk et al. 2013). In addition, phytase supplementation at 500 FTU kg^{-1} in the NC 2 diet improved ($P < 0.05$) metacarpal ash weight and bone breaking strength comparable to pigs fed the PC, and there was no additional benefit on bone ash to feeding 2000 FTU kg^{-1} phytase (Table 4). These results indicate the diets were deficient enough in Ca and avP to create a reduction in metacarpal ash concentration or breaking strength. Phytase supplementation at 500 FTU kg^{-1} improved bone ash comparable to the PC indicating this novel phytase was efficacious at hydrolysing phytate and providing a source of avP and Ca. This has been previously reported in growing pigs fed P deficient diets with *E. coli* or fungal phytases (Jendza et al. 2005; Brana et al. 2006). Phytate is present in vegetable ingredients (Ravindran et al. 1994), is poorly digested by monogastric animals, and reduced mineral availability (Schlegel et al. 2010) and protein and energy digestibility (Liao et al. 2005) in growing pigs. In the current trial, supplementation of phytase at 2000 FTU kg^{-1} in pigs fed the NC 2 diet improved ($P < 0.05$) overall G:F compared with the PC. The current results and previously published results in younger pigs (Walk et al. 2013) indicate supplementation of superdoses of phytase, above 500 FTU kg^{-1}, may improve growth performance or feed efficiency due to hydrolysis of phytate and improvements in overall nutrient utilisation or efficiency, rather than through the provision of P. In conclusion, reducing dietary avP and Ca in the current diet did not negatively influence growth performance. However, bone breaking strength or bone ash weight were reduced and may be more sensitive to reduced dietary Ca and P. Phytase supplementation at 500 FTU kg^{-1} improved bone ash weight and breaking strength comparable to the PC. Phytase supplementation at 2000 FTU kg^{-1} improved G:F compared with the PC, but had no further impact on metacarpal parameters indicating further improvements in performance may be associated with phytate destruction rather than P provision.

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