EAAP 2004

Session: P4.8
Tuesday September 7, 2004
Presenter: Michael Azain (mazain@uga.edu)

The interaction of dietary phosphorous and phytase on nutrient digestibility and bone characteristics in growing pigs.

M. J. Azain[1] and M. R. Bedford[2],

[1]University of Georgia, Athens, GA and [2]Zymetrics, Golden Valley, MN, USA.

Abstract

A total of 108 pigs (initial wt =9 kg) were randomly assigned to one of 9 dietary treatments with 2 barrows and 2 gilts per pen in a 3 x 3 arrangement of treatments to examine the main effects of dietary phosphorous (P) and phytase. There were 3 levels of available P (0.13, 0.28 and 0.42%). Calcium was maintained at 0.85%. Within each level of P, diets were supplemented with 0, 500 or 12,500 U /kg feed of a novel E. coli phytase expressed in Pichia pastoris. Diets were fed for 28 d. Apparent nutrient digestibility was determined using chromic oxide as a marker during the last week of the trial. Metatarsal bones were isolated for determination of bone strength and mineral content. Phytase improved growth rate, efficiency and phosphorous digestibility in the 0.13% P diet, but had little effect on the diets with 0.28 or 0.42% P. However, effects of phytase on bone parameters were noted at all levels of dietary P. Increasing diet P and addition of phytase improved bone ash and bone strength. Addition of P or phytase to the diet increased bone content of P and Mg, but decreased Fe and Cu content. There was no effect of diet on bone Ca content. The results suggest effects of phytase in diet s with adequate P that may be explained by removal of the anti-nutritional effects of phytin.

Introduction

Nitrogen and phosphorous are the two nutrients that are of greatest concern as environmental pollutants from livestock operations. Nitrogen can be controlled by proper balancing of diets to avoid excess protein. Phosphorous is more of a challenge because much of the phosphorous in diets, particularly those fed to swine and poultry is not available to the animal. Much of the phosphorous in seeds such as in corn or soybean meal is in the form of phytic acid and is undigestible. Because of this, inorganic forms of phosphorous are added to diets, typically in the form of calcium phosphate salts. There are several approaches available to improve the efficiency of phosphorous utilization and to decrease phosphorous excretion. These include use of low phytic acid grains (Veum et al., 2002), transgenic pigs expressing the enzyme phytase (Golovan et al., 2001) and use of phytase as a dietary supplement (Simons et al, 1990; Knowlton et al., 2004). Of these, only the latter is practical on a large scale at this time. Currently, there are several phytases available. The effects of the fungal phytases are well established. In the pig, the benefits of phytase on growth or bone mineralization, plateau in the range of 800-1200 U phytase / kg of diet. In contrast, it seems that E. coli phytases show greater efficacy, with responses reported at higher levels (Azain and Bedford, 2004). There is evidence from direct comparisons that the bacterial phytases have greater efficacy than the fungal sources of the enzyme (Adeola et el., 2004; Augspurger et al., 2003). The objective of this study was to examine the effects of phytase on growth, nutrient digestibility and bone strength and mineralization in nursery pigs fed diets with varying levels of available phosphorous.

Materials and Methods

The protocol for this study was approved by the University of Georgia Institutional Animal Care and Use Committee. The study was conducted in the Large Animal Research Unit using pigs from the University herd. The pigs (n=104)used were the progeny of PIC lines and had an average initial weight of 8.6 kg. The pigs were approximately 6 weeks of age at the start of the study and were weaned at 3 wk of age and fed a nursery diet that met or exceeded the NRC recommendations for pigs of this age (NRC, 1998).

The study was conducted as a 3 x 3 factorial design with main effects of available phosphorous level (0.13, 0.28, 0.42%) and phytase (0, 500 and 12,500 U/kg), resulting in 9 dietary treatments. The facility that was used had 26 pens, thus there was one missing cell, resulting in 3 pens per diet with the exception of the 0.28% P, 0 U phytase diet which had only 2 pens. There were 2 barrows and 2 gilts in each pen.

The composition of the diets used is shown in Table 1. Diets were corn and soybean meal based and were formulated to contain 1.11% lysine. The 3 basal diets contained varying amounts of available phosphorous which was achieved by altering the dicalcium phosphate inclusion. Calcium content was maintained at 0.71 % by the addition of limestone. Phytase (Zymetrics Quantum) was added to the basal diets to result in phytase activities of 0, 500 and 12,500 U /kg of phytase activity. Diets were fed ad libitum for 28 days and intake and body weight of the pigs was monitored at weekly intervals.

Fecal samples were collected during the last week of the study for determination of apparent nutrient digestibility. Fecal samples were composited from each pen, freeze-dried and analyzed for nitrogen, energy and minerals (ICP). Digestibility was determined using chromic oxide as a marker.

Pigs were euthanized after obtaining the final body weight. The rear legs were removed for later isolation of the metatarsal bones. Bone weight, length, ash and mineral profile were determined on one metatarsal and bone strength (Instron Universal Testing Machine, Model 1122 with a 5500R Series system interface) was determined on the other.

Results were analyzed using the PROC GLM procedure in SAS. Pen was considered the experimental unit for growth performance and digestibility. The individual pig was considered the experimental unit for the bone determinations. The model included the main effects of dietary phosphorous and phytase and their interaction. All results are shown as LS means.

Results and Discussion

The diets were submitted for phytase analysis by an independent laboratory. Results clearly showed the expected increase in phytase activity for the 500 and 12, 500 U diets with 0.13 and 0.42% P, but the phytase activity was not confirmed for the 0.28% P diets and is being repeated (data not shown). However, an in vitro assay for P release was able to distinguish all 3 levels of phytase at each level of available P (data not shown).

In designing the study, it was our intention that the 0.28% available P diet be adequate for the pigs. The NRC recommendation for available P is 0.32% for pigs in the weight range of 10-20 kg and 0.23% for pigs from 20-50 kg. The pigs used in the study were in the range of 8.5-20 kg and thus the 0.28% diets would best be labeled as marginal for dietary P. The 0.42% diets were above the requirement.

Growth performance results are shown in Table 2. There were significant main effects of dietary P and phytase for body weight, gain, intake and efficiency. The interaction of main effects was also significant for all except intake. In general, the interaction can be accounted for by a diminished response to added phytase in the higher P diets. Pig fed the low P diets had poorer growth than those in the marginal or high P, which were not different from each other. The addition of phytase improved growth rate and final body weight, with the effects being more obvious in the low P diets than in the marginal or high P. Phytase addition had no effect on performance in pigs fed the marginal P diets, but did result in a numerical improvement in gain ($P < 0.10$) and final body weight ($P < 0.01$) in pigs fed the high P diet.

In the low and high P diets, addition of phytase increased intake, but the response was not linear. The effect was less obvious in the maringal P diets. Efficiency (Feed:gain) was improved with the addition of P to the low P diet and within dietary P by the addition of phytase. The effects of phytase were stronger in the low P diet than in the high.

The effects of phytase on protein and energy digestibility (Table 3) were not consistent across dietary P levels, resulting in significant statistical interactions for the main effects. There was no main effect of dietary P on protein or energy digestibility. Calcium and phosphorous digestibilities were improved by both dietary P and phytase and as with growth performance, the effects of phytase were less obvious in the high P diet than in the low. As a result of the addition of phytase to the low P diet, fecal P concentration was reduced dramatically from greater than 2% on a dry matter basis to less than 1% with the highest level of phytase. This improvement in P digestibility is greater than that observed with fungal phytases (Knowlton et al., 2004) and is in agreement with our previous observation (Azain and Bedford, 2004).

Bone weight, strength and ash content were improved by both dietary P and phytase addition (Table 4). As for other responses, the effects of phytase were greatest in pigs fed the low P diet. Bone weight, percent ash, and strength were improved 47, 34 and 111% in pigs fed 12,500 U phytase as compared to no phytase in the low P diet. The corresponding improvements seen in pigs fed the high phytase diet were: 12, 4 and 30%.

Bone mineral profile is also shown in Table 4. The percent calcium was not affected by dietary treatment. Percent P and Mg was increased by both dietary P and phytase, with no interaction noted. In contrast, iron and copper content decreased. Zinc was not affected by phytase, but was reduced as dietary P increased. While the effects of phytase in the marginal P diet were less obvious for growth performance, they were clearly evident for the bone parameters.

As expected, the effects of phytase were much more dramatic in the low P diets than in the high. Nevertheless, effects of phytase were observed in the high P diets. For example, in the low P diet, phytase improved growth rate 22%, while in the high P diet, the improvement was 6%. Phosphorous digestibility was improved by 185 and 9%, respectively in the low and high P diets. Bone strength was improved 111 and 30% in low and high P diets. Thus, there is an additional response seen in diets that are meeting the available P requirement. This suggests that perhaps phytase is removing some other negative effect.

A final point to be emphasized is the impact of E. coli phytase on fecal phosphorous content. In the low P diet, fecal P concentration was 2.38% in pigs fed the unsupplemented diets. P content was reduced to 0.86% by the addition of phytase. This improvement in P digestibility is generally greater than reported for fungal phytases. While these high levels of phytase supplementation may not be economically practical at present, they may become feasible as environmental restrictions increase. While not tested in this study, it is likely that performance can be maintained at optimal levels in pigs fed diets with no inorganic phosphorous, but with supplementation of high levels of phytase. Others have reported reductions in fecal P (Mroz et al., 1994). There is a need to determine P balance to obtain more accurate determinations of P retained.

Literature Cited

Adeola, O., J. S. Sands, P. H. Simmins, and H. Shulze. 2004. The efficacy of an Esherichia coli-derived phytase preparation. J. Anim. Sci. 82: 2657-2666.

Augsperger, N. R., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Effficacy of an E. coli phytase expressed in yeast for releasing phytate-bound phosphorous in young chicks and pigs. J. Anim. Sci. 81: 474-483.

Azain, M. J., and M. R. Bedford. 2004. The effects of high levels of phytase on nutrient digestibility, growth performance and bone characteristics in growing pigs. J. Anim. Sci 82: (abstract).

Golovan, S. P., R. G. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Widerkehr, D. J. Barney, C. Plante, J. W. Pollard, M. Z. Fan, M. A. Hayes, J. Laursen, J. P. Hjorth, R. R. Hacker, J. P. Phillips, and C. W. Forsberg. 2001. Pigs expressing salivary phytase produce low-phosphorous manure. Nature Biotechnology 19: 741-745.

Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase supplementation of low phosphorus growing finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Animal Sci. 75:3174-3186.

Knowlton, K. F., J. S. Radcliffe, C.L. Novak, and D. A. Emmerson. 2004. Animal management to reduce phosphorous losses to the environment. J. Anim Sci. 82: (E. Suppl.) E173-195.

Lei, X.G., Ku, P.K., Miller, E.R., Yokoyama, M.T., and Ullrey D.E. 1995. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. J. Anim. Sci. 72: 139-143.

Mroz, Z., A.W. Jongbloed, and P.A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci. 72: 126-132.

NRC, National Research Council, 1998. Nutrient requirements of swine. 10th ed. National Academic Press, Washington, DC.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R.F., And Verschoor, G.J. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540.

Veum, T. L., D. R. Ledoux, D. W. Bollinger, V. Raboy, and A. Cook. 2002. Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs J. Anim Sci. 80: 2663-2670.

Table 1. Basal Diet Composition[1]

Available P, %:	0.13	0.28	0.42
Ingredients:			
Corn	62.75	62.40	62.09
Soybean meal	31.52	31.57	31.62
Fat	3.00	3.00	3.00
Salt	0.56	0.56	0.56
Dicalcium Phosphate	0.13	0.92	1.65
Limestone	1.54	1.05	0.59
Vitamin Premix	0.15	0.15	0.15
Minerals	0.25	0.25	0.25
Chromic Oxide	0.10	0.10	0.10
Calculated Analysis			
ME, kcal/kg	3450	3445	3440
CP, %	20.73	20.72	20.71
Lysine, %	1.11	1.11	1.11
Ca, %	0.71	0.71	0.71
Total P, %	0.41	0.57	0.72
Available P, %	0.13	0.28	0.42

[1] Phytase premix (2500 U/g) was added to each basal diet to create the 500 and 12,500 U phytase/kg diets.

Table 2. Effect of Dietary Phosphorous and Phytase on Growth Performance on Growing Pigs

Available P:	0.13			0.28			0.41			SEM	P Value		
Phytase:	0	500	12,500	0	500	12,500	0	500	12,500		Phos	Phytase	P*P
Body Weight													
Day 0	8.65	8.65	8.57	8.70	8.67	8.69	8.71	8.61	8.63	0.05	NS	NS	NS
Day 7	9.80	10.20	10.66	10.37	10.80	10.96	10.75	10.65	10.74	0.15	0.002	0.01	0.11
Day 14	12.40	12.94	14.17	13.92	14.24	14.16	14.00	14.33	14.49	0.27	0.002	0.01	0.06
Day 21	15.13	16.89	17.71	17.31	17.86	17.78	17.89	18.33	18.86	0.40	0.0002	0.005	0.20
Day 28	17.59	20.14	21.37	21.89	21.85	21.32	21.63	22.72	22.85	0.45	0.0001	0.005	0.01
Gain, g/d													
Day 0-14	268	307	400	373	398	391	378	409	418	18	0.002	0.005	0.05
Day 14-28	371	514	514	569	543	512	545	599	591	37	0.005	0.20	0.20
Day 0-28	319	411	457	471	471	451	462	504	502	16	0.0001	0.005	0.005
Intake, g/d													
Day 0-14	552	608	602	611	669	634	605	656	669	19	0.005	0.01	NS
Day 14-28	813	985	964	922	1020	923	991	1030	1003	37	0.05	0.02	NS
Day 0-28	682	796	783	766	844	778	798	843	832	24	0.01	0.005	NS
Feed: Gain													
Day 0-14	2.06	1.98	1.51	1.64	1.68	1.62	1.60	1.62	1.60	0.07	0.001	0.01	0.01
Day 14-28	2.20	1.92	1.88	1.69	1.88	1.82	1.82	1.72	1.71	0.11	0.02	NS	NS
Day 0-28	2.14	1.94	1.71	1.64	1.79	1.73	1.73	1.67	1.66	0.05	0.0001	0.02	0.005

Results represent 3 pens of 4 pigs each on each diet (except Diet 4 0.28P, phytase which has 2 pens).

Table 3. Effect of Dietary Phosphorous and Phytase on Nutrient Digestibility

Available P:	0.13			0.28			0.41			SEM	P Value		
Phytase:	0	500	12,500	0	500	12,500	0	500	12,500		Phos	Phytase	P*P
Digestibility, %													
Protein	75.0	82.0	72.2	73.1	73.2	79.9	82.6	72.5	75.5	2.7	0.74	0.83	0.004
Energy	82.7	84.6	76.1	77.6	77.6	82.6	85.0	80.3	78.9	1.3	0.19	0.05	0.0002
Phosphorous	44.4	46.1	68.0	22.2	43.5	63.3	62.8	48.1	68.5	3.7	0.001	0.0001	0.0001
Calcium	22.4	44.1	62.8	58.7	58.2	68.4	66.9	63.4	69.8	4.7	0.005	0.004	0.25
Fecal Concentration													
Phosphorous, %	2.38	1.94	0.86	2.28	1.80	1.21	2.09	2.31	1.42	0.11	0.05	0.0001	0.004
Calcium, %	3.66	2.94	1.74	2.19	1.81	2.11	1.71	2.54	1.82	0.25	0.005	0.02	0.002

Results are least squares means from 3 pens of pigs in each treatment (Except for the 0.28% P, 500 U diet which had 2 pens). Feces were collected on 3 days during the 4th week of feeding and pooled prior to analysis.

Table 4. Effect of Dietary Phosphorous and Phytase on Bone Characteristics

Available P:	0.13			0.28			0.41			SEM	P Value		
Phytase:	0	500	12,500	0	500	12,500	0	500	12,500		Phos	Phytase	P*P
Bone Wt, g	3.58	4.43	5.28	4.82	5.55	5.56	5.18	5.64	5.81	0.28	0.0001	0.0001	0.31
Ash, g	0.98	1.23	1.93	1.56	1.96	2.18	1.93	2.18	2.23	0.10	0.0001	0.0001	0.01
Ash, %	27.3	27.9	36.6	32.6	35.5	39.4	37.2	38.8	38.6	1.0	0.0001	0.0001	0.002
Length, cm	5.30	5.65	5.75	5.66	5.72	5.72	5.65	5.59	5.72	0.11	0.34	0.11	0.22
Width, mm	10.1	10.7	11.6	11.4	11.4	11.7	11.8	11.8	11.8	0.20	0.0001	0.02	0.04
Bone Minerals													
Calcium, %	38.1	39.1	38.1	38.0	38.5	38.8	38.6	38.5	39.5	0.5	0.54	0.40	0.37
Phosphorous, %	17.63	18.21	18.26	18.12	18.39	18.68	18.52	18.54	18.74	0.20	0.002	0.02	0.68
Sodium, %	1.21	1.17	1.15	1.06	1.17	1.16	1.18	1.10	1.01	0.05	0.15	0.57	0.005
Potassium, %	0.74	0.66	0.64	0.67	0.61	0.64	0.61	0.62	0.62	0.04	0.42	0.48	0.05
Magnesium, %	0.62	0.65	0.77	0.69	0.75	0.79	0.75	0.76	0.80	0.02	0.0001	0.0001	0.18
Iron, ppm	193	178	147	141	139	119	142	129	126	11	0.0001	0.01	0.04
Copper, ppm	11.4	7.5	6.4	6.3	5.8	4.7	7.3	5.2	4.7	1.2	0.005	0.01	0.07
Zinc, ppm	404	377	365	354	331	322	320	335	356	11	0.0001	0.39	0.81
Bone Strength													
Displacement	10.2	10.0	7.7	8.8	8.3	7.9	7.5	7.7	7.5	0.45	0.0001	0.004	0.03
Strain, %	24.5	24.4	17.6	20.5	19.8	18.3	17.3	17.3	17.4	1.3	0.0001	0.01	0.03
Load, kgf	11.7	15.4	24.7	21.8	29.9	34.2	28.1	37.9	36.0	2.7	0.0001	0.0001	0.38
Stress, kgf/mm^2	0.40	0.48	0.76	0.66	0.91	0.94	0.83	1.09	0.88	0.09	0.0001	0.01	0.10

Results are based on determination for 8-12 pigs per dietary treatment.
Load is the force in kg / mm^2 at breakage.
Stress represents internal resistance to deformity.
Strain represents percentage of deformity.