EFFECT OF PHYTASE SUPPLEMENTATION ON MINERAL AVAILABILITY AND PLASMA BIOCHEMICAL PARAMETERS IN CROSSBRED PIGS

K. Shyama[1] and K. M. Syam Mohan[2]
Department of Animal Nutrition,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur, Kerala

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Abstract

A feeding trial for a period of 114 days was conducted using 36 weaned cross bred (Large White Yorkshire x Desi) piglets to assess the effect of phytase supplementation on mineral availability and plasma biochemical parameters. The animals were divided into three groups (as uniformly as possible with regard to age, sex and weight and animals of each group were allotted randomly to six pens with two piglets in each pen) and were fed with three experimental rations, T1- control ration (NRC 1998) containing 0.6 per cent calcium and 0.3 per cent phosphorus(control), T2 - control ration without any mineral supplements and with 750 units of phytase/kg feed and T3-Control ration without phytase and mineral supplementation. A digestibility trial was conducted at the end of the experiment to determine the availability of minerals of the experimental diets. Availability of Ca, P and Mn was higher while that of Mg was lower for T2 than that of the control ration and Zn was also lower for T3 than that of T1 ration. Blood samples were collected at the beginning and at 16th week of the experiment and on analysis of the results there was no difference (P>0.05) in plasma Ca, P, Mg Zn, Cu, Mn and ALP activity between pigs fed the three experimental rations. It can be concluded that supplementation of phytase at 750 units / kg feed to cross bred pigs resulted in better availability of Ca, P and Mn with decreased feed cost per kg gain.

Key words: *Phytase supplementation, mineral availability, pigs*

India produces 481 thousand tones of pork per year which account for 17.4 per cent of total meat produced in the country (FAO, 2010). Swine rearing is an enterprising livelihood of farm sector owing to the fast growth, better feed conversion efficiency and prolificacy of pigs and pork can fill up the large gap between the availability and requirement of meat in the country. Swine are fed mainly with cereal grains which are generally low in Ca while P is present mainly as phytate P with low availability. Phytates also form complexes with Ca, Mg and other cations reducing their availability in monogastric animals Mc Donald *et al*. (2002).

Under field condition, pigs are reared on kitchen/hotel wastes alone without any mineral or vitamin supplementation. Dietary addition of phytase in pigs is documented to release a large portion of naturally occurring phosphorus from phytate P and this can greatly reduce the amount of inorganic phosphorus to be added in the pig diet. It is also used as a tool to reduce phosphorus excretion and thus reduce the environmental pollution. Rearing of pigs without any mineral supplementation, which is the common practice among pig breeders of the state, will result in reduced growth performance and bone abnormalities. Hence, an

1. Assistant Professor
2. Associate Professor & Finance Officer, KVASU

investigation was undertaken to study whether phytase supplementation can alleviate negative effects of mineral deficiency by assessing the effect of phytase supplementation on mineral digestibility, and blood parameters along with cost of production in cross bred pigs.

Materials and Methods

Experimental Animals

Thirty six Large White Yorkshire x Desi weaned piglets (18 castrated males and 18 females) belonging to the Centre for Pig Production and Research, Mannuthy were randomly selected and were divided into three groups, as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into six pens with two piglets in each pen. They were randomly allotted to the three experimental treatments.

Housing and Management

All animals were dewormed before the start of the experiment. Each replicate was housed in separate pen in the same shed with concrete flooring and facilities for feeding and watering. The animals were washed every day in the morning before 10AM and stalls were cleaned twice daily before morning and afternoon feeding. All the animals were fed with the respective ration in mash form and restricted feeding was followed throughout the experimental period. They were allowed to consume as much feed as they could, within a period of one hour. Balance of feed was collected and weighed before the next feeding. Clean drinking water was provided in all the pens for twenty four hours throughout the experimental period.

Experimental Rations

The animals were fed with standard grower ration up to 50 kg body weight and finisher ration from 50 to 70kg body weight formulated as per NRC (1998), to contain 18 percent CP and 3200kcal of ME /kg of feed and 16 per cent CP and 3200kcal of ME / kg of feed, respectively. The three experimental rations were T1-Control ration containing 0.6 per cent Ca and 0.3 per cent P, T2- Control ration without any mineral supplements and with 750 units of phytase / kg feed and T3- Control ration without phytase and mineral supplementation. The ingredient and chemical composition (AOAC, 1990) of the starter and finisher rations are furnished in Tables 1 and 2 respectively. Piglets of the three groups were maintained on the three experimental rations T1, T2 and T3 from weaning till they attained slaughter weight of 70 kg. Daily feed intake was recorded.

Table 1. Ingredient composition of experimental starter and finisher rations

Ingredients	Starter rations			Finisher rations		
	T1	T2	T3	T1	T2	T3
Yellow maize, kg	70	70	70	76	76	76
Soya bean meal, kg	29.4	29.4	29.4	23.5	23.5	23.5
Salt, kg	0.5	0.5	0.5	0.5	0.5	0.5
Lysine, kg	0.1	0.1	0.1			
Total	100	100	100	100	100	100
To 100 kg of the above mixture added						
Dicalcium phosphate, kg	1.7	-	-	1.7	-	-
Shell grit, kg	0.6	-	-	0.6	-	-
Zinc oxide, g	75			75		
Indomix AB_2D_3, g[1]	25	25	25	25	25	25
Rovi BE, g[2]	25	25	25	25	25	25
Phytase, g[3]	-	30	-	-	30	-

[1]Indomix A, B_2, D_3, K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A- 40,000 IU, Vitamin B_2-20mg, Vitamin D_3-5000 IU and Vitamin K- 50mg, per gram

[2] Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B_1-4 mg, Vitamin B_6-8mg, Vitamin B_{12}-40mg, Niacin-60 mg, Calcium pantothenate-40 mg, Vitamin E- 40 mg per gram.

[3]Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram

Table 2. Chemical composition of grower[1] and finisher rations, %

Parameter	Grower Rations			Finisher rations		
	T1	T2	T3	T1	T2	T3
Dry matter, %	92.80	92.30	92.24	88.85	88.53	87.83
Crude protein, %	18.43	18.48	18.37	16.15	16.32	16.63
Ether extract, %	2.8	2.54	2.58	2.58	2.79	2.64
Crude fibre, %	3.57	3.23	3.14	3.49	3.48	3.32
Total ash, %	6.35	5.19	4.82	5.7	3.42	3.30
Nitrogen free extract, %	68.85	70.56	71.09	71.68	73.99	74.11
Acid insoluble ash, %	1.74	1.22	0.97	0.76	0.66	0.57
Calcium, %	0.75	0.2	0.19	0.75	0.20	0.20
Phosphorus, % (total)	0.57	0.25	0.24	0.56	0.26	0.25
Magnesium, %	0.33	0.24	0.24	0.34	0.24	0.25
Zinc, ppm	262.03	36.39	37.81	336.47	44.18	42.90
Copper, ppm	9.59	9.52	9.03	9.40	9.05	9.80
Manganese, ppm	13.79	13.44	12.98	13.16	13.58	12.44

[1] *On DM basis*

Plasma Biochemical Parameters

Blood samples were collected at the beginning and at 16th week of the experiment in clean dry test tubes using heparin as anticoagulant. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma for analyzing Ca, Mg, Mn, Cu and Zn by Atomic Absorption Spectrophotometer (Perkin Elmer 3110) using hollow cathode tubes, and phosphorus by Phosphomolybdate method, using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane. The alkaline phosphatase enzyme (ALP) activity in plasma samples was estimated using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane.

Digestion Trial

A digestibility trial was conducted at the end of the experiment to determine the availability of minerals of the experimental diets. Before the commencement of the actual collection period, animals were subjected to a preliminary period of three days when they were fed the same quantity of the feed. Total quantities of the faeces voided were collected for three days as and when they were voided, uncontaminated with feed, dirt or urine. Samples were collected on each day from each animal were weighed and representative samples were taken after thorough mixing. These samples were placed in double lined polythene bags, labeled and kept in deep freezer until further analysis. The representative samples of feed offered during the collection period were also taken each day and were pooled and sub samples were taken for the analysis. Faecal samples of each animal were taken after pooling the samples of three days of digestibility trial and taking subsamples. The feed and faecal samples were analyzed for proximate principles (AOAC, 1990) and minerals such as Ca, Mg, Mn, Cu and Zn were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer 3110) after wet ashing using nitric acid and perchloric acid (2:1). Phosphorus contents of the feed and faecal samples were analyzed by colorimetry (Vanado-molybdate method, AOAC, 1990) using Spectrophotometer (Spectronic 1001 plus, Milton Roy, USA).

Economics of Gain

Cost of feed per kg body weight gain of pigs maintained on the three dietary treatments were calculated and compared.

Statistical Analysis

Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Least Significant Difference (LSD) test.

Results and Discussion

Plasma Biochemical Parameters

The results of plasma biochemical parameters of animals belonging to the three groups are given in Table 3.

Table 3. Plasma biochemical parameters of animals [1]

Treatments	Calcium mg/dl	Phosphorus mg/dl	Magnesium mg/dl	Zinc ppm	Copper ppm	ALP U/L
T1	10.95	5.22	3.29	0.66	1.65	609.83
T2	10.56	5.58	3.79	0.68	1.69	552.67
T3	10.67	5.46	3.70	0.69	1.64	550.67
Pooled SE	0.21	0.08	0.12	0.04	0.04	12.32

[1] Mean of six values

Table 4. Availability of minerals[1] of three experimental rations, %

Treatments	Minerals					
	Ca	P	Mg	Zn	Mn	Cu
T1	52.03^{a}	50.07^{a}	68.15^{a}	65.77^{a}	72.13^{a}	67.60 $^{(NS)}$
T2	64.68^{b}	58.05^{b}	64.20^{b}	64.93^{ab}	76.96^{b}	70.68$^{(NS)}$
T3	59.88^{bc}	52.45^{ab}	62.80^{bc}	61.78^{bc}	73.16^{ac}	71.09$^{(NS)}$
Pooled SE	1.59	1.37	0.81	0.78	0.62	1.17
P	0.00	0.04	0.01	0.08	0.00	0.44

Mean of six values a, b, c Means with different superscripts within each column differ, NS Non-significant

Calcium, Phosphorus and Magnesium

The statistical analysis of the data on plasma Ca and P revealed no significant (P>0.05) difference among pigs belonging to the treatment groups T1, T2 and T3. Pigs of all the three groups showed normal plasma Ca level irrespective of phytase or Ca supplementation. Moreira *et al.* (2003) also observed that plasma concentrations of Ca P or Ca: P ratios were not significantly affected by dietary phytase supplementation in pigs while, Martinez *et al*. (2004) could observe higher plasma P concentration in pigs fed phytase supplemented diets. Farzinpour *et al.* (2011) showed that supplementation of broiler diets with commercial phytase increased levels of non phytate phosphorus and decreased serum Mg. However, no change was observed in plasma Ca concentration.

Zinc

Statistical analysis of the data on plasma zinc concentration indicated that the values were similar in all the three treatment groups (P>0.05). Phytase supplementation to rations without mineral supplementation has no effect on the plasma Zn concentration in pigs. Adeola *et al*. (1995) reported that plasma Zn concentration increased when phytase was added to the diet containing no supplemental Zn, but plasma Zn concentration was not affected by phytase, when the diet was supplemented with Zn.

Copper

The animals belonging to the three dietary groups had similar (P>0.05) plasma Cu concentrations, the values being 1.02, 1.13 and 1.19ppm initially and 1.65, 1.69 and 1.64ppm at 16th week. Murry *et al.* (1997) observed no consistent effect of dietary microbial phytase for serum Cu concentrations in pigs fed supplemental phytase which is in agreement with that of the present study.

Alkaline Phosphatase

In the present study statistical analysis of the data revealed that the difference between groups was nonsignificant (P>0.05). Guggenbuhl *et al.* (2012) in their work on the efficacy of a novel microbial phytase expressed in *Aspergillus oryzae* on the performance and phosphorus utilization in swine observed that blood alkaline phosphatase concentrations were restored to their normal physiologic levels when the piglets were supplemented with phytase in comparison to non supplemented diets.

Availability of Minerals

The data on the availability of different minerals are shown in Table 4.

Calcium

In the present study the percentage availability of Ca for the three rations T1, T2 and T3 were 52.03, 64.68 and 59.88 respectively.

Statistical analysis of the data showed that Ca availability of the rations T2 and T3 was higher (P<0.01) than that of ration T1 while there was no difference between rations T2 and T3. Johnston *et al.* (2004) observed improved Ca availability and absorption along with phytase supplementation in pigs. Brana *et al*. (2006) observed that there was no effect of Ca:P ratio or Natuphos enzyme level on the quantity of Ca absorbed in pigs in a comparative study between the two enzymes Natuphos and Phyzyme. However, they noted that Ca absorption increased in response to Phyzyme additions and Veum *et al*. (2006) also observed improved Ca digestibility and absorption along with phytase supplementation in pigs. In contrast to the findings of the present study, Guggenbuhl *et al.* (2012) in their work on the efficacy of a novel microbial phytase on the performance and phosphorus utilization in swine observed that the enzyme increased the utilisation and reduced faecal excretion of Ca in a dose dependent manner and results suggested that the animals could tolerate phytase and the enzyme significantly increased Ca availability in piglets and pigs.

Phosphorus

Data on percentage availability of P of the three experimental rations T1, T2, T3 were 50.07, 58.05 and 52.45 respectively. On statistical analysis it was observed that rations supplemented with phytase showed higher (P<0.05) P availability than that of T1 and T3. Increased P availability and retention as a result of phytase supplementation was also reported by Veum *et al*. (2006) in pigs.

Brana *et al*. (2006) observed that there was no effect of Ca:P ratio or Natuphos enzyme level on the quantity of Ca absorbed in pigs in a comparative study between the two enzymes Natuphos and Phyzyme. They further concluded that phytase supplementation improved the apparent availability of P over the control by 44 per cent and 22 per cent with Phyzyme and Natuphos, respectively. Veum *et al*. (2006) observed that pigs fed barley-based diets containing low P supplemented with 2,500 or 12,500 PU/kg of *E. coli* phytase had greater apparent absorption (per cent) of P, Ca, and Mg than pigs fed the positive control diets. Guggenbuhl *et al.* (2012) in their work on the efficacy of a novel microbial phytase expressed in *Aspergillus oryzae* on the performance and phosphorus utilization in swine observed that the enzyme increased the utilisation and reduced faecal excretion of P in a dose dependent manner.

Magnesium

Availability of Mg from experimental rations T1, T2 and T3 were 68.15, 64.20 and 62.81 per cent respectively. Statistical analysis of the data showed lower (P<0.05) Mg availability for rations T2 and T3 than that of T1. In contrast, with the observations in present study Veum *et al*. (2006) observed that pigs fed barley based diets containing low P supplemented with phytase had greater per cent apparent absorption of Mg than those fed the positive control diets while Viswanathan *et al*. (2007) could not note a better apparent digestibility of Mg in citric acid and phytase supplemented groups of Large White Yorkshire pigs, which is in accordance with the result of the present study.

Zinc

From the data presented in Table 4, it could be seen that the per cent availability of Zn for the experimental rations T1, T2, and T3 were 65.77, 64.93 and 61.78 respectively. On analysis of the data statistically there was significant difference (P=0.08) between the treatment groups with regard to Zn digestibility. On further analysis of the data it was evident that T3 ration was showing significantly lower (P<0.05) Zn digestibility than that of T1. Ration T2 had similar Zn digestibility (P>0.05) to that of T1 indicating that phytase supplementation improved digestibility of Zn from the ingredients used in the ration. Veum *et al*. (2006) on the contrary, observed that addition of *E. coli* phytase did not increase the apparent percentage absorption of Zn in pigs. In agreement to the results of the present study, Viswanathan *et al*. (2007) observed a higher apparent digestibility of Zn for rations supplemented with citric acid and phytase.

Copper

The per cent Cu availability of the three experimental rations of the present study were similar (P>0.05) the values being 67.60, 70.68 and 71.09 respectively for rations T1, T2 and T3. Veum *et al*. (2006) observed that addition of phytase did not increase the apparent percentage absorption of Cu which

is in agreement with that of the present study. However, Kies *et al.* (2006) observed increased availability of Cu in weaner pigs with increasing phytase levels in digestible P deficient corn-barley soyabean meal diets while Viswanathan *et al*. (2007) observed a better apparent digestibility of Cu in citric acid supplemented groups of pigs.

Manganese

The per cent availability of Mn recorded for the three treatment groups was 72.13, 76.96 and 73.16 respectively. The statistical analysis of the data revealed higher (P< 0.01) Mn availability for ration T2 than that of T1 and T3, indicating that phytase supplementation increased the availability of Mn in pigs fed rations without any added minerals. In concurrence with the present study Viswanathan *et al*. (2007) observed a better apparent digestibility of Mn in citric acid plus phytase supplemented groups.

Economics of Gain

Phytase was supplied by Polchem Hygiene Laboratories Pvt Ltd, Pune at the rate of Rs. 350 /kg. Cost of feed per kg body weight gain of pigs maintained on the three dietary treatments was Rs. 65.73, 59.90 and 62.44 respectively. Feed cost per kg gain was lower (P< 0.01) for T2 than that of T1. It can be concluded that phytase supplementation of rations resulted in decreased feed cost per kg gain compared to control diet.

From the overall results, it can be concluded that supplementation of phytase in pigs will alleviate the negative effects of mineral deficiency in cross bred pigs to a great extent by increasing the availability of minerals such as Ca, P and Mn. However, long-term feeding experiments are to be carried out before making recommendations.

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