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THE EFFECT OF SUPPLEMENTAL FUNGAL PHYTASE ON THE PERFORMANCES AND BONE CHARACTERISTIC OF PIGLETS

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The 31 day long trial included 24 Yorkshire piglets divided into three groups. Before weaning, the piglets were given the experimental diets in a 7-day adjustment period. During this period as well as for 22 days following, the piglets were fed on diets containing 22% CP and then diets containing 20% CP for the remaining 19 days. The desired level of phosphorus in the diet for the control group and experimental group III was achieved by the addition of dicalcium phosphate, while the diet for group II was without added dicalcium phosphate. In addition the, diets for groups II and III were enriched with crude phytase (produced by growing an isolate of Aspergillus awamori) in an amount sufficient to provide 800 FU/kg. Performance and state of health were monitored during the trial and at the end the piglets were killed for collection of bone samples.

Growth performance was improved by adding microbial phytase. Ash and calcium content in the tibia showed no statistical differences between the groups, while content of phosphorus was significantly increased in both groups fed added phytase in the diet. The length and width of the tibia and the intensity of its shadow indicated normal physiological processes in bone formation and ossification. The best ossification was obtained in the first experimental group, based on almost invisible nutrition channels inside the thick shadow of compacta, as well as the homogenous appearance of tibiae spongiosa in the mid diaphysis area.

Key words: phytase, piglets, performance, bone

INTRODUCTION

Phytate phosphorus, which is largely unavailable to nonruminant animals, accounts for approximately 60 to 80% of total phosphorus in most grains and other feedstuffs of plant origin (Obreleas, 1973). The poor availability of dietary phosphorus has been ascribed to the presence of phytic acid (myo-inositol-hexa-kis dihydrogen phosphate), as well as due to insufficient phytase enzyme activity in the gastrointestinal tract of single-stomached animals. Depending on the vegetable feed, the digestibility of phytin phosphorus varies between zero (e.g. corn) and 40% (e.g. wheat) in the pig. The major part of this phosphorus fraction is thus excreted unused and leads to the presence of large amounts in pig manure (Cromwell and Coffey, 1991). Phytic acids form complexes with other minerals, also reducing their availability (Brink et al., 1991, Pallauf et al., 1994a, b).

The use of phytase (EC 3.1.3.8.), an enzyme preparation that results in the stepwise removal of phosphorus from the phytate molecule, was reported to increase phosphorus availability in diets for pigs (Nasi, 1990). Phytase hydrolyzes phytate to phosphoric acid and inosinic acid and releases the orthophosphate group from the phytate molecule (Gibson and Ullah, 1990). There is also some evidence that phytase will enhance the utilization of other nutrients (Ketaren et al., 1993; Mroz et al., 1994). Based on improved phosphorus availability, phytase increased growth rates and performance in piglets, and these results have been validated by many authors (Jongbloed et al., 1992; Cromwell et al., 1993;1995; Lei et al., 1993 a, b, c).

The presence of phytase in some feed, e.g. wheat, barley, may enhance the utilization of phytate phosphorus (Pointillart et al., 1987). Phytase of plant origin appears to retain its activity when added to animal diets (Gibson and Ullah, 1990), as well as phytase from *E. coli* when fed to animals (Nelson et al., 1971). Recently, a microbial phytase from *Aspergillus* has been used to improve the availability of phytate phosphorus in corn-soybean meal diets for pigs (Simons et al., 1990; Pallauf et al., 1994; Yi et al., 1996).The purpose of this study was to evaluate the effectiveness of dietary fungal phytase from *Aspergillus awamori* on the performance of piglets.

MATERIAL AND METHODS

Animals. A total of twenty-four Yorkshire piglets from the Agroekonomik Institute swine herd were used in this study. Equal numbers of barrows and gilts from three litters were included and littermates were balanced across the treatments as much as possible. Piglets were group housed in pens on elevated, plastic-coated expanded metal floors in an environmentally controlled room. Each pen was equipped with a pair of nipple waterers and a stainless feeder.

Design of the experiment. The trial lasted from weaning till 76 days of age. The day after partus piglets from three litters were randomly assigned to treatments within outcome groups based on sex and weight. The piglets were weaned at 35 days of age and an average weight of about 10 kg. Before weaning, the piglets were given a 7-day adjustment to the experimental diets. During the adaptation period as well as for 22 days following, piglets were fed diets containing 22% CP and then diets containing 20% CP for the remaining 19 days. At the end of the trial the piglets were killed for collection of bone samples.

Diets. All groups of piglets were fed with a commercial mixture (Inshra, Padinska Skela) which consisted of standard feeds (Table 1) and contained enough nutrients to provide the requirements for the category (NRC, 1988; AEC, 1993). The desired level of phosphorus in the diets for groups I and III was achieved by the addition of dicalcium phosphate, while the diet for the group II was without added dicalcium phosphate. In addition, diets for groups II and III were enriched with crude phytase by adding a fungal extract to the starter or grower, in amount sufficient to provide 800 FU/kg. First in the morning, piglets were offered a small quantity of feed with added solution, and later were fed *ad libitum*.

The phytase was produced by growing an isolate of *Aspergillus avvamori* in cornstarch-based medium under optimal conditions for enzyme production (Shieh and Ware, 1968). The material obtained after sterilization was diluted in physiological saline solution to provide 80 FU/ml. The phytase activity was assayed according to the procedures of Engelen et al. (1994).

Table 1. Composition of complete feed mixtures, %

Ingredient, %	Group			Group		
	C	O-I	O-II	C	O-I	O-II
			Composition, %			
Maize, raw	54.7	55.0	54.7	53.5	53.7	53.5
Wheat	-	-	-	15.0	15.0	15.0
Soybean meal, solvent	24.0	24.0	24.0	20.0	20.0	20.0
Fish meal	6.0	6.0	6.0	6.0	6.0	6.0
Skim milk powder	10.0	10.0	10.0	-	-	-
Animal fat	3.0	3.0	3.0	-	-	-
Aflafla meal	-	-	-	3.0	3.0	3.0
Dicalcium phosphate	1.0	-	1.0	1.0	-	1.0
Limestone, ground	-	0.7	-	-	0.8	-
Iodized salt	0.3	0.3	0.3	0.5	0.5	0.5
Vitamin-mineral, premix	1.0	1.0	1.0	1.0	1.0	1.0
Molds solution		+	+		+	+
			Calculated analysis, %			
ME.MJ/kg	14.93	14.98	14.93	13.00	13.00	13.00
Grude protein	22.26	22.29	22.26	19.45	19.47	19.45
Lysine	1.40	1.40	1.40	1.10	1.10	1.10
Meth.+Cyst.	0.72	0.72	0.72	0.63	0.63	0.63
Tryptophan	0.31	0.31	0.31	0.28	0.28	0.28
Calcium	0.92	0.91	0.92	0.84	0.86	0.84
Phosphorus,total	0.80	0.62	0.80	0.74	0.56	0.74
Phosphorus, available	0.44	0.32	0.44	0.38	0.26	0.38
Phosphorus, phytin	0.21	0.21	0.21	0.24	0.24	0.24

Data and sample collection. Performance and state of health were monitored during the trial. Body weight and pen feed consumption were measured after birth and then on the 35th, 57th and 76th day of the trial. From the measured data, the average daily gain and feed:gain ratio were calculated.

At the end of the trial the piglets were killed for collection of bone samples. The tibia and ulna from both sides were cleaned of all tissues and frozen in sealed plastic bags till analysis. The bones were radiographically analyzed (Mobigraf 2) and then used for determination of ash, as well as content of Ca and P. Bone ash was obtained in a muffle furnace at 600 ^{o}C for 24 h. Total P concentration was assayed photometrically by a standard procedure (AOAC, 1990) and Ca was determined by flame atomic absorption spectrophotometry (Perkin Elmer 3300).

Statistical analysis. All data obtained were statistically processed (Snedecor and Cochrarn, 1971) and an appraisal was made of the significance of differences in mean values among the groups of piglets.

RESULTS AND DISCUSSION

Piglets of the control group had harmonious conformation and properly developed bone and muscle tissue, a vivid temperament and were in good condition. All clinical findings were normal. Their appetite was good, and droppings were normally formed. No disturbances of health were observed in the experimental groups. No leg problems associated with the low-phosphorus diet were observed.

The results for body weight and weight gain are summarized in table 2. Both parameters were increased by adding phytase and were independent of dietary phosphorus level. Average body weights in all experimental groups were very similar during the adjustment period (1st and 35th days), without significant differences. Later on the average body weights showed significant differences associated with nutritive treatment. Adding phytase increased the body weights in both experimental groups and the effects were more visible in younger than in older piglets. Although body weights can be valuable for estimation of feed quality and nutritive value, daily weight gain is a more reliable parameter. Higher weight gains of the experimental groups were observed through out the trial, but significant differences (p 0.05) were recorded only in the first phase of the feeding trial.

Adding phytase to the diet resulted in almost equal feed intake (Table 3) during the trial, although it was somewhat higher in younger piglets. Feed : gain ratio can be assumed as the derivation of daily gain and feed consumption, or, at the bottom line, one of the best markers of feed quality and productivity. By adding phytase to the diet, overall feed:gain ratio was increased by 8.1-13.0%. As before, more visible effects were seen in the first phase of the feeding trial.

Table 2. Body weight* and weight gain* of piglets

Trial, days	Group		
	C	O-I	O-II
		Body weight, kg	
1	1.89±0.13	1.94±0.16	1.95±0.22
35	10.34±1.22	10.76±0.73	10.43±1.10
57	15.05±1.24[a]	17.04±2.16[b]	16.91±2.52
76	26.56±2.51	29.21±3.37	28.15±1.05
		Weight gain, g/day	
1-35	0.241±0.03	0.252±0.02	0.243±0.03
35-57	0.214±0.02[a]	0.285±0.08[b]	0.295±0.11[b]
57-76	0.606±0.12	0.645±0.11	0.592±0.09
35-76	0.396±0.06	0.450±0.07	0.432±0.04

*Values expressed as X±SD
[a, b, c] Mean values within columns with unlike superscripts were significantly different (p.05, LSD test)

Table 3. Feed intake and feed:gain ratio

Trial, days	Group			Group		
	c	O-I	O-I	C	O-I	O-II
	Feed intake, kg/day			Feed:gain ratio, kg		
35-57	0.335	0.423	0.395	1.56	1.48	1.34
57-76	0.917	0.874	0.922	1.51	1.36	1.56
35-76	0.637	0.663	0.639	1.61	1.40	1.48

Phytase was added to the diets containing 0.44% available phosphorus (aP) to evaluate any possible extra-phosphorus effects of the enzyme. The results revealed that such an effect was seen during the first 24 days of the trial and piglets fed the positive control diet with added phytase grew faster (295 vs. 214 g) and were more efficient in feed utilization (1.34 vs. 1.56 kg) than piglets fed the positive control diet without phytase. The results of this study clearly demonstrate the beneficial effects of microbial phytase addition to a low P maize-soybean meal based piglet diet. Growth performance was improved by adding microbial phytase to the diet and this is in agreement with other studies which have demonstrated increased daily gains and decreased feed: gain ratio (Beers and Jongbloed, 1992; Kornegay and Qian, 1996; Yi et all., 1996; Radcliffe and Kornegay, 1998).

The content of ash and calcium (Table 4) found in the tibia of the piglets showed no significant differences, while the content of phosphorus was significantly increased in both groups of piglets fed with added phytase in the diet. A better response of bone phosphorus percentage occured in pigs fed 0.44/0.38% aP than in those fed 0.32/0.26 aP. A partial explanation is that the Ca:aP ratio in the latter diet was wider than in the former. The wider Ca:aP ratio may impair the utilization of phosphorus and phytase (Qian et al., 1996). Another possible reason is that the amounts of available phosphorus in low-P diets after phytase addition were still deficient, whereas the amounts of available phosphorus in higher-P diets after phytase addition were close to the actuel requirements of piglets which suggested that the recommended levels (NRC, 1988; AEC, 1993) may be somewhat lower than necessary. Radcliffe and Kornegay (1988) found that the amount of phosphorus excreted per day decreased approximately 24.6% by adding 500 FU/kg diet, and thus retention of phosphorus increased. Similar results have been reported in many studies citing 25-50% reduction of phosphorus excretion (Jonbloed et al., 1992; Lei et al., 1993b; Kornegay and Qian, 1996; Zi et al., 1996). Thus, pigs fed a diet which contained 0.62% tP and 500 FU/kg should have a similar performance to pigs fed a diet with approximately 0.78% of total phosphorus. Therefore, adding 800 FU/kg diet in our trial should lead to somewhat better results. On the other hand, the activity of microbial phytase produced in our laboratory is probably lower than commercial enzyme produced by a leading world manufacturer.

Table 4. Content of ash, calcium and phosphorus in tibia of piglets, %

	Group		
	C	O-I	O-II
Ash	50.67±1.48	50.03±2.30	50.13±2.36
Calcium	16.11±1.26	15.67±0.76	15.54±0.52
Phosphorus	$5.94\pm0.33^{a,x}$	6.77 ± 0.60^{b}	7.65±0.43

^{b}*Values expressed as X±Sd
^{a,b,c} Mean values within columns with unlike superscripts were significantly different ($p<0.05$, LSD test)
^{x,y,z} Mean values within columns with unlike superscripts were significantly different ($p<0.01$, LSD test)

The results obtained for the length and width of the tibia and the intensity of its shadow (Table 5) indicated normal physiological processes in bone formation and ossification. Numerical differences between the groups did not indicate possible disorders. Although the control group had somewhat longer and wider bones, especially in relation to the second expermental group (p 0.01), which indicates the faster deposition of minerals, the calciferous shadow was narrower than in the experimental groups (p 0.05).

Contours of tibiae compacta (Figure 1) in the control group of piglets were discontinuous, sharp and cut through with light zones of nutrition channels, going from the periosteum to the medullar channel. In the mid diaphysis area of the tibia, the compacta had a woolly and tufty look. Contours of the compacta in the first group were extremely sharp and evident, while the contours of nutrition channels were almost invisible. In the second group contours were sharp and emphasized, but in the central diaphysis area with a partially tufty look. Hawers and Folkmans channels were more visible than in the first group of piglets.

Table 5. Morphometric parameters of radiographically analyzed tibia of piglets

	Group		
	C	O-I	O-II
Length, cm	12.10±0.37[X]	11.90±0.045	11.47±0.26[y]
Width, cm	1.47±0.12[X]	1.60±0.08	1.70±0.08[y]
Calciferous shadow, cm	1.67±0.47[a]	2.50±1.08[b]	2.44±0.42

*Values expressed as XSd

a, b, c Mean values within column with unlike superscripts letters were significantly different ($p<0.05$, LSD test)

x, y, z Mean values within column with unlike superscripts letters were significantly different ($p<0.01$, LSD test)

The cortical calciferous shadow passing around the tibial epiphyses was not especially emphasized in any group, being the most visible and completely covering epiphyses in the first experimental group. The calciferous shadow of the corticalis in the control group was toothlike and discontinuous, even missing at some parts, while in the second group it was clear, but thinner than in the first group.

In the medullar channel shadow of the control group reduction of bone trabeculae and lamellae could be seen. In the first group spongious tissue was homogenous, without traces of rarefaction, while medullar channels of the second experimental group were mildly marbled.

Based on the analyses of the first line macrostructure of the tibia and ulna, it could be concluded that the best ossification was obtained in the first experimental group, based on the almost invisible nutrition channels inside the thick shadow of compacta, as well as the homogenous appearance of tibiae spongiosa in the mid diaphysis area.

Phosphorus is an essential element required by piglets for optimal growth and bone development. Much of the P in piglet diets is unavailable to the piglets because it is bound as phytate P. Approximately 66% of the P in maize and 61% of the P in soybean meal is complexed in phytate P (Ravindran et al., 1994). Therefore, producers have to add large amounts of inorganic P to piglet diets in order to meet the requirements of the piglets, but this adds costs and results in increased P excretion which could lead to environmental pollution problems. According to the NRC (1988), a 10-20 kg piglet requires an 18% CP containing 0.32% available phosphorus. A maize-soybean meal based diet formulated to contain 18% CP contains 0.38% phosphorus. Theoretically, if all of this P was available it would be enough to meet the piglet requirement for phosphorus. However, since approximately 85% of the P in maize and 75% of the P in soybean meal is unavailable to the piglets, the diet actually contains only 0.07% available P and a highly available inorganic source of phosphorus has to be supplemented to the diet.

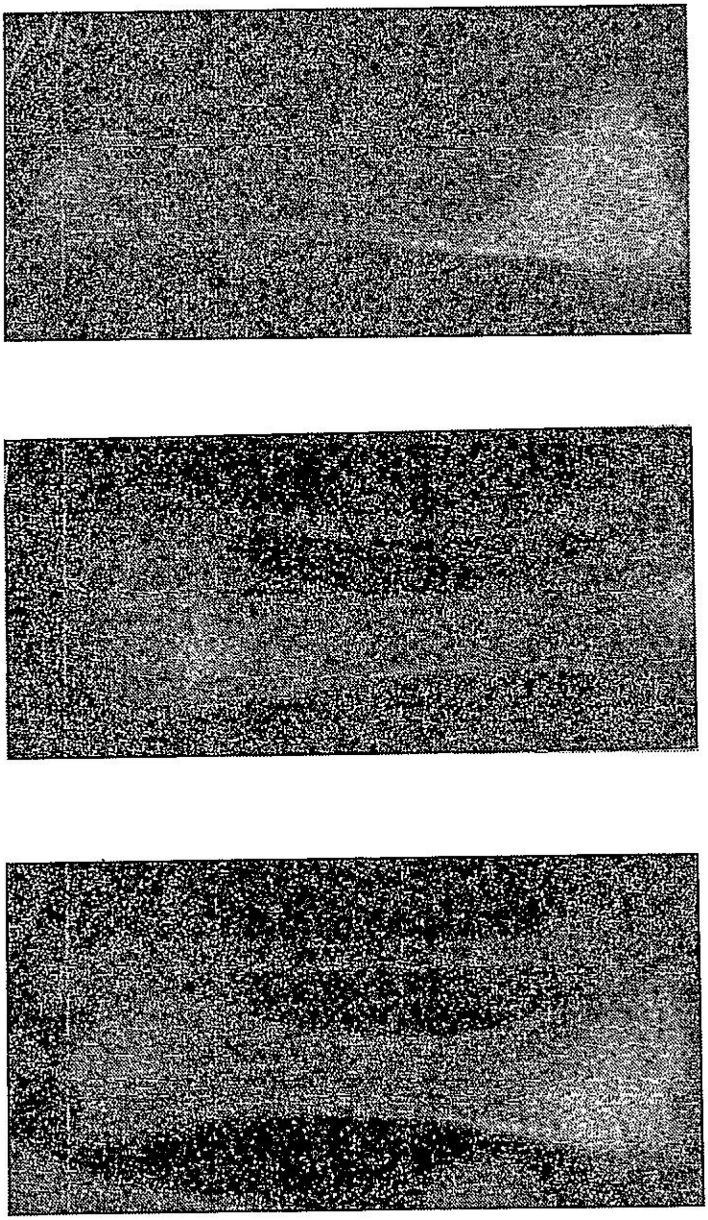

Figure 1. X-ray radiograph* of piglets tibia and ulna
*Anterior-posterior position
C- control group
O-I, O-II - experimental group

Ruminants can utilize phytate P because microbes within the rumen produce the enzyme phytase which hydrolyzes the phytate molecule releasing the bound phosphorus. However, pigs and poultry only have trace amounts of phytase in their digestive tract. The potential to release P bound by phytate through addition of exogenous phytase was shown nearly 30 years ago (Nelson et all., 1971). However, an affordable method of producing microbial phytase has not been available until recently. Many studies have documented the ability of microbial phytase to enhance P digestibility and decrease P excretion (Jongbloed et al., 1992; Kornegay et all., 1996). With commercially produced phytase now available to swine producers it is essential that accurate equivalency values of phytase for phosphorus be derived. This needs to be done firstly to minimize the cost of adding inorganic sources of phosphorus, and secondly to minimize the amount of phosphorus excreted in pig manure. The results of this study clearly demonstrate the beneficial effects of microbial phytase produced in our laboratory and added to a low P maize-soybean meal based piglet diet.

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UTICAJ FITAZE MIKROBIOLOŠKOG POREKLA NA PROIZVODNE REZULTATE I KARAKTERISTIKE KOSTIJU PRASADI

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SADRŽAJ

Ogled je izveden na 24 odbijene Jorkšir prasadi podeljenih u tri grupe, a trajao je 31 dan. U pripremnom periodu od 7 dana pre odbijanja, kao i tokom naredna 22 dana prasadu su hranjena smešom sa 22% proteina, a zatim smešom sa 20% proteina do kraja ogleda. Planirani nivoi fosfora u I i III grupu postignuti su dodavanjem dikalcijum fosfata, dok su smeše za ishranu II grupe prasadi bile bez dodatog dikalcijum fosfata. Smešama za K i O-II grupu prasadi dodata je sirova fitaza (izolovana iz gljivica *Aspergillus awamori)* u količini koja je obezbedila 800 FU/kg hrane. Tokom ogleda praćeni su proizvodni rezultati i zdravstveno stanje, a na kraju ogleda izvršeno je rtvovanje prasadi u cilju uzimanja uzoraka kostiju za predviđena ispitivanja.

Proizvodni rezultati su bili poboljšani korišćenjem fitaze mikrobijalnog porekla. Razlike u sadraju pepela i kalcijuma tibije nije se staistički razlikovao, dok je sadraj fosfora signifikantno rastao u grupama sa dodatom fitazom mikrobijalnog porekla. Rezultati morfometrijskih i radiografskih ispitivanja ukazalu su na normalne fiziološke procese osifikacije kostiju. Na osnovu dobijenih rezultata najbolje izraen proces osifikacije kostiju bio je u prvoj oglednoj grupi prasadi.