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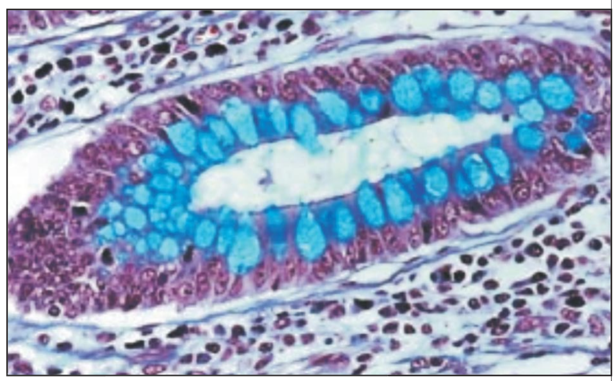

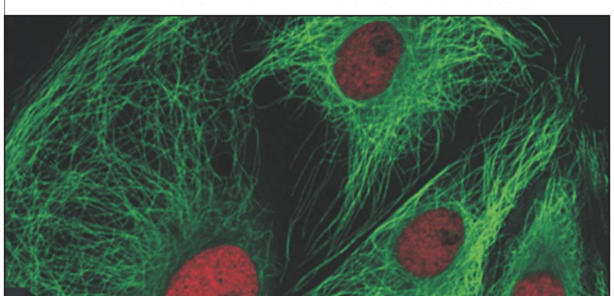

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Nutrition and Physiology

Effect of dietary exogenous phytase supplementation in growing and fattening pigs

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Abstract. *Three balance feeding trials were performed with 3 groups male castrated pigs during the starter, grower and finisher periods. The animals were housed in specially designs individual cages that allowed for separate collection of urine and faeces. Each trial comprised 5 days preliminary and 5 days experimental period. Pigs were fed compound feeds according to their genotype and age. The first groups received compound feed without exogenous phytase. The feed of the second group was supplemented with the Bulgarian enzyme preparations Optiphos at 0.01 %, and the feed of group III - with the same amount of the enzyme preparation Natuphos. The results showed that the inclusion of exogenous phytase in compound feeds for pigs permitted to decrease the amounts of used dicalcium phosphtate by 3 to 7 times. This reduced the amount of total dietary phosphorus by 27-30 % and the cost of feeds. The utilization of phytase improved the absorption of phosphorus by 28-34 % and decreased its total output by 38-45 %, without any effect upon nitrogen retention. The performed trials did not shown any significant difference between the effects of both exogenous phytases used: Optiphos and Natuphos.*

Keywords: phytase, phosphorus availability, digestibility, nitrogen balance, nitrogen retention, pigs.

Introduction

The main feeds for pigs are relatively rich in total phosphorus (grain cereals 3.0-3.5 g/kg, meals 6.5-9.5 g/kg, barn 10.0-11.0 g/kg) (Todorov et al., 2007), but about 60-70% of it is under the form of phytates (Cheryan, 1980). Phytates are salts of phytic acid with bivalent cations, proteins and carbohydrates, with a very limited digestibility in pigs (Jongbloed et al., 1987; Sandberg, 2002). The cause is the deficiency of the enzyme phytase that is needed for destruction of phytate molecule (Reddy et al., 1982; Jongbloed et al., 1993). Phytase could only be produced by microorganisms (moulds, bacteria and yeasts), growing on nutrient media or dwelling the alimentary tract of animals (mainly herbivores). The phytase produced by the latter is known as 3-phytase (EC 3.1.3.8) (Jongbloed et al.,1995).

Monogastric animals possess insignificant phytase activity due to intestinal mucosa (Jongbloed et al., 1992; Jongbloed et al., 1999). Ruminants, by virtue of the microflora in forestomachs, digest and utilize well the phosphorus from plant feeds. Plants also possess phytase activity. Plant phytase is determined as 6-phytase (EC 3.1.3.26), but it is encountered at relatively low concentrations (Wyss et al., 1999). The deficiency of endogenous phytase in monogastric animals is the cause for poor phytate phosphorus utilization. According to Cromwell et al. (1993) only about 16% of phosphorus in corn and about 38% от phosphorus from soybean meal – principal ingredients in swine rations, are digested and utilized. The other amount could not be utilized by animals and is excreted with faeces, thus contaminating the environment.

The ability of pigs to utilize nutrients chelated in phytate complexes depends on endogenous phytase activity of feed ingredients on one parts, and on the supplementation of rations with exogenous phytase preparations. At present, there is a variety of commercial microbial phytase preparations that are in either dry or liquid form. Liquid phytase is widely used in pelleted feeds. The optimal pH for microbial phytases' activity ranges between 2.5 and 5.5 (Simons et al., 1990). In experiments with pigs Jongbloed et al. (1992) established that the hydrolysis of phytates from rations containing microbial phytase occurred mainly in the stomach (43%) and considerably less in small intestine (7%). Thus only half of phytates were hydrolyzed by microbial phytases that could be attributed to the restricted period of stay of feed in the stomach. Nevertheless, the effect of exogenous phytase utilization in the nutrition of pigs was relatively high.

Since 1990, multiple trials with exogenous phytases have been performed to determined their effect upon the growth intensity of animals, the consumption and utilization of feed, the apparent digestibility and the absorption of minerals and energy from diets. In a large review, Jongbloed et al. (2006) have summarized the results of numerous experiments upon the effect of microbial phytase in swine. The authors concluded that in most experiments, the inclusion of phytase had a positive impact for growth intensity and feed intake. Similar conclusions were made by Sands et al. (2001), Brady et al. (2002), Shelton et al. (2004) Sands and Kay (2009). The effect of phytase upon the utilization of feed is relatively constant (Brady et al., 2002; Barrera et al., 2004; Shelton et al., 2004). This, according to Campbell et al. (1995) and Selle et al. (1996) could be explained by the higher digestibility of protein, amino acids and energy.

The most consistent influence of microbial phytases was observed upon digestibility and absorption of phosphorus (Sands et al., 2001; *Sands, 2004;* Fan et al., 2005; Patraš et al.,2006). During the hydrolysis of phytates under the action of phytase, considerable amounts of available phosphorus are released. This permits to reduce the phosphorus supplements without affecting the balance of rations. Low dietary phosphorus and the better utilization of plant phosphorus reduce the excretion of this macroelement and therefore decrease environmental pollution. According to Cromwell et al. (1993), the excretion of phosphorus for one kg weight gain was

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reduced by about 40% over 20 years. The inclusion of phytase in compound feeds for pigs has a positive effect on the retention and excretion of other minerals from the phytate complex Ca, Zn, Cu, Mn etc. (Matsui, 2002; Kies et al., 2005; Valencia and Chavez, 2009).

The effect of utilization of exogenous phytase depends on numerous factors the amount and source of phytates (Dekker et al.,1992), feed composition (Eeckhout and De Paepe, 1992; Dungelhoef et al.,1994) the addition of acidifiers (Jongbloed et al.,2000), the physiological state and rearing conditions (Mroz et al.,1994, Kemme et al.,1997) etc. That is why reported literature data are rather various.

The present experiment aimed to investigate the effect of two kinds of phytases - Optiphos and Natuphos supplemented to compound feeds for growing and fattening pigs. Optiphos is a new preparation manufactured in Bulgaria, but it is still not used in our country.

Figure 1. Specially designed individual cages

Material and methods

Five balance feeding trials were performed with castrated male pigs. The experiments were performed with 9 pigs divided into 3 groups. Each trial consisted of 5-day preparation and 5-day experimental periods. During the experimental period, the urine and faeces of animals were collected and the exact amounts of consumed feed and water were recorded. The trials were carried out in the Experimental Farm of the Faculty of Agriculture, Trakia University, Stara Zagora.

Experimental equipment

During the balance trials, the pigs were housed in specially designed individual cages (Figure 1) that allowed for easy separation and collected of urine and faeces. The feed was offered in feeders that limited its spill and water – in ordinary waterers mounted on the left side of pens.

The feeding was done with compound feeds according to animals' genotype and age (Table 1). Group I received compound feed without phytase supplement. The feed of group II was supplemented with the Bulgarian enzyme preparation Optifos at 0.01%, and that of group III – with the same amount of the enzyme preparation Natuphos.

The recipes of compound feeds were formulated on digestible amino acids basis. For this purpose, the total amino acids content was determined by means of amino analyzer (T 339M, Mikrotehna – Praha), and digestible amino acids were calculated on the basis of analysis results and coefficients of digestibility recommended by Degusa (AminoDat Platinum version 3.0 – 2007). For rations balancing, synthetic amino acids – L-lysine, DL-methionine and L-threonine were used.

Table 1. Ingredients of compound feed for pigs used in experiments

Ingredients, %	Starter period			Grower period			Finisher period		
	Cont-rol	Opti-phos	Natu-phos	Cont-rol	Opti-phos	Natu-phos	Cont-rol	Opti-phos	Natu-phos
Corn	28.58	30.33	30.14	25.28	27.03	26.90	18.63	21.94	21.92
Wheat	30.00	30.00	30.00	30.00	30.00	30.00	18.00	18.00	18.00
Barley	10.00	10.00	10.00	10.00	10.00	10.00	40.00	40.00	40.00
Soybean meal	18.50	18.20	18.20	14.20	14.00	14.00	7.00	6.00	6.00
Sunflower meal	3.70	3.70	3.70	7.00	7.00	7.00	6.00	6.00	6.00
Wheat bran	-	-	-	7.00	7.00	7.00	5.00	4.00	4.00
Fish meal	3.70	3.70	3.70	-	-	-	-	-	-
Sunflower oil	1.90	1.20	1.30	3.20	2.40	2.50	2.60	1.80	1.80
Lysine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.04	0.04	0.04	0.05	0.03	0.04	0.03	0.03	0.03
Threonine	0.15	0.14	0.15	0.11	0.11	0.10	0.07	0.08	0.08
Salt	0.33	0.33	0.33	0.28	0.28	0.28	0.24	0.24	0.24
Limestone	1.10	1.33	1.40	0.68	0.97	1.00	0.90	1.00	1.00
Dicalcium phosphate	1.20	0.22	0.23	1.40	0.37	0.37	0.73	0.10	0.12
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase*	-	0.01	0.01	-	0.01	0.01	-	0.01	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.00	100.0

* Phytase was supplemented to the diet of pigs according to the experimental design. In group II, the preparation Optiphos 5000 (Huvepharma, Peshtera, Bulgaria) was used, whereas in group III - Natuphos 5000 (BASF, Ludwigshafen, Germany).

The nutrition of animals was ad libitum and feed was placed twice daily – at 8.30 AM and 4.30 PM. Its amount was adjusted in the preliminary stage of the trials in a manner such that a small excess was always available. The daily amount of feed for each pig was weighed in polyethylene bags, and approximately half of the amount was placed in the morning, the other half – in the afternoon. The remainders of feed was collected every morning prior to placing the feed. For each animal, they were placed in individual bags. At the end of each trial, the total amount of feed that was not consumed was weighed and a sample for analysis was taken out.

During the trials, the ambient temperature in the premise was comfortable - 21-22 ^{0}C.

Collection of excretion

Faeces were collected in the morning, prior to feeding (Figure 2). Two faecal samples were obtained from the total amount on a daily basis. The first sample (5% of total amount) was used for determination of nitrogen in fresh faeces and the second (10% of total amount) was dried in a drier at 60-65 ^{0}C. Fresh faeces were stored in refrigerators at -17^{0}C until analyzed. By the end of each 5-day period, faeces of each animal were mixed together and after homogenization, an average sample was taken for analysis. Dried faeces of each pig were collected in individual polyethylene bags. By the end of the trial, after grinding with a laboratory mill with sieve diameter of 1 mm, an average sample was taken for analysis

Figure 2. Faecal samples collected

The excreted urine was collected in plastic containers (Figure 3) containing 100-200 ml 10 % H_2SO_4 (5% of urine volume). Every morning, the volume of urine was measured and 10% of it was collected for analysis. Urine samples of each pig were collected in individual plastic bottles and kept in a refrigerator until analyzed.

Figure 3. Plastic containers for urine samples.

Analyses and calculations

The total nitrogen content in compound feeds, and N output in faeces and urine were determined by the Kjeldahl method. The general chemical analysis of feeds and dried faeces was performed as per routine methods (AOAC - 2005). The content of Ca and P in feeds and faeces were assayed according to Ibrishimov et al.(1974), and Ca and P in urine – by means of commercial kits of Chema diagnostica, Italy on a semi-automated biochemistry analyzed MINDRAY BA-88. For calculation of assimilable nitrogen, obtained as an effect from the addition of phytases, data matrices from the manufacturers of the respective phytase product were used. All samples were run in duplicate.

Productivity traits (daily weight gain, feed consumption and feed conversion) and data from balance trials) retention and excretion of nitrogen and phosphorus) were calculated for each animal for every 5-day period. The statistical analysis of data was performed by the Statistica 6 software.

Controlled parameters:

1. Live body weight – individual weighing of pigs after 12-hour deprivation of food and water.
2. Feed intake, kg/day
3. Water intake, l/day
4. Amount of faeces, kg/day
5. Amount of urine, l/day
6. Retention and excretion of nitrogen and phosphorus

Results and discussion

The data from Table 1 about the ingredients of compound feeds showed that the balance of needs for protein and amino acids during the different periods was achieved by utilization of four types of protein components – soybean meal, sunflower meal, wheat bran and fish meal (only during the starter period). For reduction of the total amount of nitrogen in the ration and the total nitrogen output with urine and faeces, synthetic amino acids (L-lysine, DL-methionine and L-threonine) were added for balancing the rations. The inclusion of phytases in compound feeds for pigs from experimental groups resulted in altered amount of minerals with particularly significant decrease in the amount of used dicalcium phosphate (from 3- to 7-fold). This reflected upon the price of compound feeds and the amounts of total phosphorus provided to animals from the different groups (Table 2). The data from the table show that compound feeds of experimental animals contained by

Table 2. Nutritive value of compound feeds used during the balance feeding trials.

Periods	Starter period			Grower period			Finisher period		
Parameters	Control	Optiphos	Natuphos	Control	Optiphos	Natuphos	Control	Optiphos	Natuphos
Metabolisable energy, MJ/kg	13.5	13.5	13.5	13.2	13.2	13.2	13.0	13.0	13.0
Crude protein, %	18.34	18.61	18.52	16.46	16.73	16.63	14.06	14.30	14.20
Crude fibre, %	2.9	2.9	2.9	4.30	4.3	4.30	4.7	4.6	4.6
Total amino acids, %									
- Lysine	1.10	1.10	1.10	0.93	0.93	0.93	0.76	0.75	0.75
- Methionine	0.33	0.33	0.33	0.29	0.29	0.29	0.25	0.25	0.25
-Methionine+cysteine	0.66	0.67	0.67	0.62	0.62	0.62	0.53	0.54	0.54
- Threonine	0.73	0.74	0.73	0.62	0.61	0.60	0.49	0.49	0.49
- Tryptophan	0.40	0.41	0.41	0.36	0.37	0.37	0.27	0.29	0.29
Digestible amino acids, %									
- Lysine	0.97	0.97	0.97	0.81	0.81	0.81	0.65	0.65	0.65
- Methionine	0.29	0.29	0.29	0.26	0.26	0.26	0.22	0.22	0.22
-Methionine+cysteine	0.57	0.57	0.58	0.53	0.53	0.53	0.46	0.46	0.46
- Threonine	0.61	0.61	0.61	0.50	0.50	0.50	0.41	0.40	0.40
- Tryptophan	0.29	0.29	0.30	0.27	0.27	0.27	0.21	0.22	0.22
Ca, %	0.77	0.77	0.77	0.67	0.67	0.67	0.60	0.60	0.60
Total P, %	0.68	0.51	0.51	0.66	0.49	0.49	0.53	0.41	0.41
Digestible P, %	0.33	0.34	0.33	0.27	0.28	0.27	0.21	0.25	0.25
Na, %	0.18	0.18	0.18	0.14	0.14	0.14	0.13	0.13	0.13

23-25% less total phosphorus compared to those of controls. The amounts of limestone also changed, but the Ca content remained constant during the different periods for the three groups.

The average feed intake from the five balance trials was 2.7 кg/day (Table 3), without significant inter-group differences. The variations with regard to water intake and amounts of faeces and urine were neither considerable. The results showed that supplementation with exogenous phytase did not have any effect on these parameters.

The data referring to nitrogen balance (Table 4) demonstrated that the average daily nitrogen intake was 63 g, without statistically significant differences between groups. Nitrogen output in faeces and urine in groups supplemented with phytase was lesser by about 5%, but differences were not considerable. On the average, the nitrogen output with faeces was 16% of dietary intake, whereas the output with urine – about 42 %. The total nitrogen output was relatively high: 35-37 g/day, that was equal to about 58-59 % of dietary intake. There was a statistically insignificant trend towards reduction of N output in groups that received phytase. Nitrogen digestibility in all trials was high – 83-84 %. Phytase supplementation had no effect on that parameter. No differences were observed with regard to nitrogen retention and absorption.

Table 3. Data for water and feed intake and urine and faeces output during the balance feeding trials using phytase

Parameters	Groups		
	Control	Optiphos	Natuphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Feed intake, kg/day	2.74±0.07	2.69±0.12	2.68±0.10
Water intake, l /day	5.62±0.28	5.95±0.39	5.22±0.32
Faeces, kg/day	1.55±0.10	1.50±0.11	1.46±0.08
Urine, l/day	2.64±0.25	2.81±0.24	2.55±0.17

The effect of compound feed supplementation with phytase was the most pronounced with regard to phosphorus balance (Table 5). The addition of phytase to compound feeds resulted in statistically significant decrease of the total phosphorus intake that was by 38 % lower than that of controls. In supplemented groups, P output with faeces was significantly lower as well. This reflected on total P output, that was almost twice lower in phytase groups. The addition of enzyme preparations improved P digestibility by more than 30 %. They confirm the findings of Sands (2004) , Fan et al.(2005), Patraš et al., (2006) that the utilization of phytase preparations improved mainly the utilization of phosphorus from plant feeds. The effect upon nitrogen retention and excretion was relatively small and inconsistent.

Apart the economic effect (reduced amount of dicalcium phosphate in feeds), the addition of enzyme preparations had a marked ecological effect. The total phosphorus output reduced by

Table 4. Nitrogen balance

Parameters	Groups		
	Control	Optiphos	Natuphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Nitrogen intake, g/day	62.99±1.26	61.44±1.85	61.73±1.75
N output in faeces, g/day	10.30±0.37	9.82±0.45	9.83±0.33
N output in urine, g/day	27.03±0.80	25.56±1.27	25.66±1.09
N absorption, g/day	52.69±1.92	51.62±2.05	51.90±2.18
Total N output, g/day	37.33±0.94	35.37±1.42	35.49±1.25
N digestibility, %	83.63±0.48	83.99±0.58	84.02±0.43
N retention, g/day	25.66±0.55	26.07±0.57	26.24±0.70
N retention, %	40.79±0.63	42.64±0.76	42.61±0.72

a,b – equal letters designate statistically significant differences

Table 5. Phosphorus balance

Parameters	Groups		
	Control	Optiphos	Natuphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
P intake, g/day	18.05±0.73[ab]	12.93±0.50[a]	13.20±0.60[b]
P output in faeces, g/day	10.64±0.44[ab]	5.93±0.27[a]	6.09±0.30[b]
P output in urine, g/day	0.88±0.11	0.80±0.10	0.76±0.14
P absorption, g/day	7.49±0.31	7.00±0.26	7.11±0.29
Total P output, g/day	11.52±0.50[ab]	6.73±0.29[a]	6.85±0.33[b]
P digestibility, %	41.06±0.68[ab]	54.15±1.04[a]	53.86±0.91[b]
P retention, g/day	6.54±0.30	6.20±0.29	6.35±0.37
P retention, %	36.23±0.85[ab]	47.93±1.05[a]	47.92±1.27[b]

a,b – equal letters designate statistically significant differences

Table 6. Calcium balance

Parameters	Groups		
	Control	Optiphos	Natuphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Ca intake, g/day	21.12±1.02	20.59±1.21	20.58±1.15
Ca output in faeces, g/day	11.99±0.84	10.66±0.90	10.38±0.80
Ca output in urine, g/day	0.54±0.07	0.62±0.07	0.66±0.08
Ca absorption, g/day	9.13±0.29	9.93±0.33	10.20±0.27
Total Ca output, g/day	12.54±0.87	11.27±0.94	11.04±0.84
Ca digestibility, %	43.49±2.60	48.86±2.43	49.66±2.57
Ca retention, g/day	8.58±0.55	9.32±0.61	9.53±0.64
Ca retention, %	40.98±2.57	45.86±2.52	46.53±2.50

38-40% on the average when compound feeds were supplemented with phytase, together with the reduced environmental pollution. According to results, the major amount of the P output (> 50% of the intake) was with faeces. Small amounts of phosphorus are excreted with urine. There was no difference in the effect of both tested phytases.

No differences were observed in the amounts of absorbed P, which were about 7 g/day for the five balance trials. The retention of phosphorus (g/day) showed neither inter-group variations. The lower intake and lower output of phosphorus in animas from experimental groups had an effect upon the retention of this element. The inclusion of phytases improved P retention by more than 30 %. Both tested phytases exhibited a similar positive effect. The supplementation of compound feeds with phytase did not influence significantly the calcium balance of pigs (Table 6).

Conclusion

The inclusion of exogenous phytase in compound feeds for growing and fattening pigs did not have a considerable effect upon feed intake and the amount of faeces and urine. The supplementation of compound feeds for pigs with phytase decreased the amount of used dicalcium phosphate from 3 to 7 times. This resulted in reduction of the total dietary phosphorus by 27-30 % and also reduced the cost of produced feeds. The addition of phytase to feeds increased phosphorus digestibility and absorption by more than 30 % and decreased its total output by 38-40 %. This way, a significant reduction of environmental contamination was achieved. From 40 to 60 % of total dietary phosphorus intake was excreted with faeces. Phosphorus output with urine was 5 to 10% of dietary intake. The supplementation of compound feeds with phytase did not influence significantly the nitrogen and calcium balance of pigs. In these experiments, no significant difference was established between the effect of both tested phytases – Optiphos and Natuphos. Enzyme preparations produced in Bulgaria could be successfully utilized in compound feeds for pigs.

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