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Agricultural Science and Technology
Faculty of Agriculture, Trakia University
Student's campus, 6000 Stara Zagora
Bulgaria
Telephone.: +359 42 699330
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Technical Assistance:
Nely Tzvetanova
Telephone.: +359 42 699446
E-mail: ascitech@uni-sz.bg

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Nutrition and Physiology

Age-related changes in mineral retention and excretion in starter and finisher pigs diets with and without exogenous phytase

A. Ilchev[1]*, G. Ganchev[1], S. Chobanova[1] , D. Kanakov[2], P. Petkov[2], I. Nikiforov[2]

[1]Department of Nutrition, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
[2]Department of Internal Non-infectious Diseases, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract. *Five balance feeding trials were performed to evaluate age-related changes in nitrogen, phosphorus and calcium utilization in growing and fattening male castrated DanBred hybrid pigs. Two commercial phytase preparations were used: Optiphos and Natuphos at a dose of 0.01 %. It was found out that with age, the amount of dietary nitrogen intake and nitrogen output in urine increased statistically significantly (P<0,05). The total nitrogen output was also higher. The retention of nitrogen decreased substantially with age. The supplementation of rations with phytase did not alter this tendency. Phytase added to feed improved phosphorus absorption by 28-34 % and reduced its total output by 38-45 %. The digestibility of calcium in pigs decreased with age, whereas its output in faeces became higher. There was no statistically significant difference between the effects of Optiphos and Natuphos.*

Keywords: phytase, age effect, digestibility, nitrogen balance, nitrogen retention, phosphorous, pigs

Introduction

The supplementation of diets for monogastric animals with microbial phytase enhances the hydrolysis of phytates and the utilization of phosphorus and other minerals (Jongbloed et al., 1992; Ketaren et al., 1993a,b; Fan et al., 2005). The use of phytase results in increased digestibility and retention of P and Ca in fattening pigs (Cromwell et al., 1993; Mroz et al., 1994; Schone et al., 1995) and reduced P (Ketaren et al., 1993b; Mroz et al., 1994; Kemme et al., 1999b) and Ca output in faeces (Schone et al., 1995; Jongbloed et al., 1996; Kemme et al., 1999b). The effect of supplementation of microbial phytase during the different fattening stages has been a subject of few investigations and the reported results are rather contradictory. In most reports, the maximum effect of phytase supplementation upon the utilization of phytin phosphorus was established for the starter period of porcine growth (Cromwell et al., 1993; Hoppe et al., 1993; Young et al., 1993; Pallauf et al., 1994; Sands et al., 2001). According to Kemme et al. (1997) the effect of phytase upon P utilization did not change throughout the entire fattening period.

The objective of this study was to establish age-related changes in nitrogen, phosphorus and calcium utilization in growing and fattening pigs, and effect of exogenous phytase added to starter, grower and finisher diets.

Material and methods

Five balance feeding trials were performed in the Experimental Farm of the Faculty of Agriculture, Trakia University with castrated male pigs at a different age. The pigs were from the DanBred hybrid and were housed in individual cages allowing individual collection of urine and faeces. Each trial consisted of 5-day preparation and 5-days experimental periods. The feeding was done with compound feeds corresponding to the genotype and the age of pigs (Table 1). In all three stages of rearing (starter, grower, finisher) the rations of the first groups were not supplemented with phytase (controls). The diets of the second groups were supplemented with exogenous phytase Optiphos at 0.01 %, and those of the third with the same amount of Natuphos.

The nitrogen content in feeds and faeces was assayed by the Kjeldahl method (BDS– ISO), and contents of calcium and phosphorus – by Ibrishimov et al.(1974). Urine Ca and P contents were analyzed with commercial diagnostic kits of Chema diagnostica, Italy on a biochemical analyzer MINDRAY BA-88. All samples were run in duplicate. Nitrogen, phosphorus and calcium balances were individually calculated for each of animals included in trials.

Results and discussion

Table 2 presents data from the intake of feed and water and for excreted urine and faeces for all stages of the nitrogen, phosphorus and calcium balance trials. Data show that the amounts of consumed feed and water increased statistically significantly with age. The amounts of excreted urine and faeces increased as well. The increase was higher from the starter to grower period and thereafter decreased. The supplementation of rations with phytase did not alter this trend.

The balance of studied minerals (Tables 3, 4 and 5) showed uniform age tendencies in all three experiments. The nitrogen intake, its output in urine and the total nitrogen output were found to increase with age. The absorption of nitrogen (g/day) did not change

* e-mail: atilbg@abv.bg

Table 1. Ingredients' content and nutritive value of used compound feeds.

Periods	Starter			Grower			Finisher		
Ingredients, %	Control	Optiphos	Natuphos	Control	Optiphos	Natuphos	Control	Optiphos	Natuphos
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	100.0	100.00
1 kg compound feed contains									
ME MJ/kg	13.5	13.5	13.5	13.2	13.2	13.2	13.0	13.0	13.0
CP %	18.34	18.61	18.52	16.46	16.73	16.63	14.06	14.30	14.20
CF %	2.9	2.9	2.9	4.3	4.3	4.3	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 +Cystin	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
Digestble 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 +Cystin	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
P total %	0.68	0.51	0.51	0.66	0.49	0.49	0.53	0.41	0.41
P available %	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

* 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).

Table 2. Feed and water intake and amounts of excreted faeces and urine by animals during the feed balance trials.

Groups/parameters	Periods		
	Starter (n = 3)	Grower (n = 6)	Finisher (n = 6)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Group I No phytase			
Feed intake, kg/day	2.26±0.03[a]	2.65±0.04[a]	3.07±0.04[a]
Water intake, l /day	3.96±0.22[ab]	6.19±0.26[a]	5.87±0.34[b]
Faeces, kg/day	1.10±0.07[a]	1.54±0.09	1.79±0.16[a]
Urine, l/day	1.89±0.05	2.69±0.34	2.98±0.50
Group II Methionine +Cystin)			
Feed intake, kg/day	2.11±0.10[a]	2.51±0.06[a]	3.16±0.09[a]
Water intake, l /day	4.50±0.77[a]	6.86±0.65[a]	5.78±0.35
Faeces, kg/day	1.05±0.14[a]	1.40±0.10[b]	1.82±0.16[ab]
Urine, l/day	1.61±0.25[ab]	3.23±0.20[a]	2.98±0.41[b]
Group III With Natuphos)			
Feed intake, kg/day	2.18±0.04[a]	2.58±0.09[a]	3.03±0.08[a]
Water intake, l /day	4.49±0.44	5.46±0.48	5.34±0.63
Faeces, kg/day	1.16±0.06[a]	1.45±0.13	1.63±0.13[a]
Urine, l/day	2.24±0.29	2.53±0.22	2.79±0.35
Average for all groups	(n = 9)	(n = 18)	(n = 18)
Feed intake, kg/day	2.19±0.04[a]	2.58±0.04[a]	3.09±0.04[a]
Water intake, l /day	4.32±0.28[a]	6.17±0.30[a]	5.66±0.26[a]
Faeces, kg/day	1.10±0.05[ab]	1.46±0.06[a]	1.74±0.08[b]
Urine, l/day	1.91±0.14[ab]	2.82±0.16[a]	2.90±0.23[b]

a,b – equal letters designate statistically significant differences

with age, but its retention decreased gradually from starter to finisher. The supplementation of compound feeds with phytases did not have any effect on these events. Data for phosphorus balance in the three groups of pigs showed that the intake of this mineral increased with age. This increase was higher in control groups, where the necessary phosphorus is supplied by the dicalcium phosphate included in rations. In experimental animals, one part of phosphorus is expected to comes from degradation of phytates by introduced enzyme preparations, and therefore the total phosphorus intake was lower as compared to controls. The phosphorus output in faeces increase slightly with age, but the output in urine did not change consistently. The supplementation of phytase reduced statistically significantly ($P< 0,05$) the phosphorus output in pigs from experimental groups. The utilization of phytase preparations increased considerably the digestibility and absorption of phosphorus, but there were no significant age-related difference in

Table 3. Nitrogen, phosphorus and calcium balance in the group of pigs non-supplemented with phytase

Parameters	Periods		
	Starter (n = 3)	Grower (n = 6)	Finisher (n = 6)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Nitrogen			
Intake, g/day	58.62±2.96[a]	62.33±1.20	65.83±2.09[a]
Fecal, g/day	9.99±0.68	9.70±0.46	11.06±0.68
Urinary, g/day	23.31±1.09[a]	26.50±0.97[b]	29.41±0.87[ab]
Absorbed, g/day	48.63±2.31	52.63±1.95	54.77±2.56
Total excreted, g/day	33.30±1.77[a]	36.21±0.66[b]	40.46±1.15[ab]
Digestibility, %	82.98±0.47	84.37±0.92	83.24±0.75
Retened. g/day	25.32±1.44	26.12±0.70	25.37±1.05
Retention, %	43.19±1.08[a]	41.89±0.52[b]	38.48±0.65[ab]
Phosphorus			
Intake, g/day	15.31±0.13	19.21±1.22	18.27±1.08
Fecal, g/day	9.00±0.26	11.15±0.78	10.95±0.64
Urinary, g/day	0.84±0.12	0.62±0.18[a]	1.16±0.15[a]
Absorbed, g/day	6.31±0.34	8.06±0.38	7.32±0.33
Total excreted, g/day	9.84±0.16	11.77±0.88	12.11±0.76
Digestibility, %	41.16±1.98	41.98±1.41	40.09±0.29
Retened, g/day	5.46±0.21[a]	7.44±0.44[ab]	6.16±0.34[b]
Retention, %	35.68±1.20	38.92±1.34[a]	33.82±0.60[a]
Calcium			
Intake, g/day	18.26±0.30	22.40±1.97	21.27±1.48
Fecal, g/day	7.21±0.39[ab]	12.72±1.04[a]	13.67±0.92[b]
Urinary, g/day	0.44±0.05	0.75±0.13[a]	0.39±0.05[a]
Absorbed, g/day	11.05±0.23	9.68±0.21	7.60±0.45
Total excreted, g/day	7.65±0.34[ab]	13.46±1.16[a]	14.06±0.94[b]
Digestibility, %	60.57±1.65[a]	42.87±1.82[a]	35.58±1.37[a]
Retened, g/day	10.61±0.18[a]	8.94±0.92	7.21±0.66[a]
Retention, %	58.13±1.32[a]	39.66±1.83[a]	33.73±1.47[a]

a,b – equal letters designate statistically significant differences

this regard. Calcium balance data indicated that the amounts of Ca intake and output increased with age, whereas its digestibility decreased regardless of the presence or not of phytase source in compound feeds. Calcium retention was significantly reduced with age. The supplementation with phytase did not alter noticeably this parameter.

The summarized data for the entire fattening period (Table 6) showed that despite the reduced amount of nitrogen in grower and finisher rations, the intake was higher as compared to starter period because of the statistically significantly higher feed intake during these fattening periods. The nitrogen output in urine and the total nitrogen output increased statistically significantly with age. The causes for enhanced nitrogen excretion could vary, but are most commonly attributed to poorer digestibility and reduced absorption. Data from the present trials demonstrated that the digestibility was not altered with age, and that nitrogen retention (g/day) was almost

Table 4. Nitrogen, phosphorus and calcium balance in the group of pigs supplemented with Optiphos

Parameters	Periods		
	Starter (n = 3)	Grower (n = 6)	Finisher (n = 6)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Nitrogen			
Intake, g/day	54.81±1.74[a]	59.26±1.90[b]	66.93±2.87[ab]
Fecal, g/day	9.56±0.61	9.31±0.45	10.45±1.00
Urinary, g/day	19.93±0.84[a]	24.03±0.99[b]	29.90±1.68[ab]
Absorbed, g/day	45.25±1.31	49.95±2.03	56.48±2.61
Total excreted, g/day	29.49±1.38[a]	33.34±1.19[b]	40.35±1.76[ab]
Digestibility, %	82.59±0.55	84.28±0.63	84.40±1.28
Retened. g/day	25.32±0.56	25.93±0.76	26.58±1.22
Retention, %	46.24±1.01[a]	43.78±0.37[a]	39.70±0.60[a]
Phosphorus			
Intake, g/day	10.68±0.41[ab]	13.35±0.80[a]	13.62±0.68[b]
Fecal, g/day	4.80±0.25[a]	5.73±0.26	6.69±0.43[a]
Urinary, g/day	0.77±0.12	0.93±0.17	0.68±0.17
Absorbed, g/day	5.88±0.24	7.62±0.31	6.93±0.28
Total excreted, g/day	5.57±0.22[a]	6.66±0.35	7.37±0.51[a]
Digestibility, %	55.15±0.59	56.78±1.51[a]	51.03±1.33[a]
Retened, g/day	5.11±0.25	6.69±0.59	6.25±0.27
Retention, %	47.85±1.11	49.79±1.97	46.11±1.51
Calcium			
Intake, g/day	16.88±0.77[ab]	21.02±2.19[a]	22.01±1.90[b]
Fecal, g/day	6.14±0.32	10.54±1.12	13.03±1.12
Urinary, g/day	0.32±0.02[a]	0.81±0.06[a]	0.57±0.10
Absorbed, g/day	10.74±0.38	10.48±0.32	8.98±0.29
Total excreted, g/day	6.45±0.33[ab]	11.35±1.17[a]	13.60±1.17[b]
Digestibility, %	63.60±1.63[a]	49.58±2.16[a]	40.77±1.06[a]
Retened, g/day	10.43±0.62	9.67±1.24	8.40±0.77
Retention, %	61.73±1.64[a]	45.61±2.45[a]	38.17±0.89[a]

a,b – equal letters designate statistically significant differences

unchanged within the experimental time period. We think that in this case, the main reason for enhanced nitrogen output was the greater nitrogen intake that was not utilized efficiently by pigs. The excessive N amounts are excreted mainly in urine. Nitrogen retention was statistically significantly reduced from starter to finisher period. That is why we assume that the allowances for level of protein in diets of DanBred hybrid could be reduced in order to improve its utilization and to prevent environmental contamination. Data referring to phosphorus intake during the grower and finisher periods showed that it was higher as compared to the starter period. The retention of phosphorus (g/day) was the highest during the grower period and decreased for the finisher period. No age-related differences in phosphorus output with faeces and urine were observed. The age of pigs had no effect on phosphorus absorption that was 44 % on the average. Age-related differences with respect to calcium balance were relatively high. With age, calcium intake and output were increased. At the same time, digestibility and absorption were reduced. The retention of this mineral decreased linearly from starter to finisher age.

Table 5. Nitrogen, phosphorus and calcium balance in the group of pigs supplemented with Natuphos

Parameters	Periods		
	Starter (n = 3)	Grower (n = 6)	Finisher (n = 6)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Nitrogen			
Intake, g/day	56.44±2.32	60.84±2.40	65.26±3.02
Fecal, g/day	9.83±0.52	9.33±0.37	10.33±0.69
Urinary, g/day	20.52±0.64[a]	25.04±1.25[b]	28.85±1.37[ab]
Absorbed, g/day	46.71±1.98	51.51±2.53	54.93±2.47
Total excreted, g/day	30.35±1.15[a]	34.37±1.22[b]	39.18±1.89[ab]
Digestibility, %	82.60±0.39	84.56±0.80	84.20±0.61
Retened, g/day	26.09±1.51	26.48±1.27	26.08±1.17
Retention, %	46.18±1.26[a]	43.45±0.64[a]	39.99±0.39[a]
Phosphorus			
Intake, g/day	1.13±0.30	13.87±1.19	13.57±0.73
Fecal, g/day	5.03±0.13	6.14±0.51	6.56±0.46
Urinary, g/day	1.10±0.41	0.70±0.27	0.65±0.11
Absorbed, g/day	6.10±0.23	7.73±0.21	7.01±0.28
Total excreted, g/day	6.13±0.54	6.85±0.66	7.21±0.42
Digestibility, %	54.81±0.66	55.44±1.59	51.80±1.33
Retened, g/day	5.00±0.32[a]	7.02±0.72[a]	6.36±0.36
Retention, %	45.35±3.64	50.43±2.34	46.87±0.90
Calcium			
Intake, g/day	17.47±0.33	21.68±2.22	21.03±1.75
Fecal, g/day	6.02±0.24[ab]	10.67±0.97[a]	12.27±0.93[b]
Urinary, g/day	0.55±0.03	0.85±0.17	0 .53±0.09
Absorbed, g/day	11.15±0.27	11.01±0.31	8.76±0.35
Total excreted, g/day	6.57±0.24[ab]	11.52±1.13[a]	12.80±0.97[b]
Digestibility, %	65.54±0.99[a]	50.17±1.86[a]	41.22±1.71[a]
Retened, g/day	10.90±0.25	10.16±1.20	8.23±0.89
Retention, %	62.39±1.05[a]	46.40±1.89[a]	38.73±1.67[a]

a,b – equal letters designate statistically significant differences

Table 6. Summarized data for nitrogen, phosphorus and calcium balance in relation to the growth stage of pigs

Parameters	Periods		
	Starter (n = 3)	Grower (n = 6)	Finisher (n = 6)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Nitrogen			
Intake, g/day	56.62±1.32[a]	60.81±1.07[b]	66.01±1.47[ab]
Fecal, g/day	9.79±0.31	9.45±0.23[a]	10.61±0.44[a]
Urinary, g/day	21.25±0.68[a]	25.19±0.65[a]	29.38±0.74[a]
Absorbed, g/day	46.83±1.32	51.36±2.07	55.40±2.56
Total excreted, g/day	31.05±0.93[a]	34.64±0.64[a]	40.00±0.85[a]
Digestibility, %	82.72±0.25	84.40±0.43	83.95±0.52
Retened, g/day	25.58±0.64	26.18±0.52	26.01±0.64
Retention, %	45.20±0.75[a]	43.04±0.35[a]	39.39±0.34[a]
Phosphorus			
Intake, g/day	12.37±0.75[ab]	15.48±0.87[a]	15.15±0.71[b]
Fecal, g/day	6.28±0.69	7.67±0.67	8.06±0.57
Urinary, g/day	0.91±0.14	0.75±0.12	0.83±0.10
Absorbed, g/day	6.09±0.28	7.81±0.25	7.09±0.29
Total excreted, g/day	7.18±0.69	8.43±0.68	8.89±0.64
Digestibility, %	50.37±2.39	51.40±1.82	47.64±1.43
Retened, g/day	5.19±0.15[a]	7.05±0.33[a]	6.26±0.18[a]
Retention, %	42.86±2.17	46.38±1.65	42.26±1.56
Calcium			
Intake, g/day	17.54±0.33[ab]	21.70±1.16[a]	21.43±0.94[b]
Fecal, g/day	6.46±0.25a	11.31±0.62[a]	12.99±0.56[a]
Urinary, g/day	0.44±0.04[a]	0.80±0.07[ab]	0.50±0.05[b]
Absorbed, g/day	11.08±0.26	10.39±0.31	8.44±0.22
Total excreted, g/day	6.89±0.24[ab]	12.11±0.67[a]	13.49±0.57[b]
Digestibility, %	63.24±1.03[a]	47.54±1.33[a]	39.19±0.98[a]
Retened, g/day	10.65±0.21[a]	9.59±0.63b	7.95±0.44[ab]
Retention, %	60.75±0.95[a]	43.89±1.34[a]	36.88±0.93[a]

a,b – equal letters designate statistically significant differences

Conclusion

The nitrogen intake and the output in urine in pigs increased significantly with age. The total nitrogen output was also higher. Nitrogen retention decreased significantly with age. The supplementation of rations with phytase did not alter this tendency. The phosphorus output in faeces ranged from 40 to 60%. This level was not significantly influenced by age. The inclusion of phytase improved phosphorus absorption by 28-34% and reduced its total output by 38-45%. This contributed a lot for diminution of environmental pollution. The calcium digestibility decrease with age and its output in faeces increased. The retention of this mineral decreased from the starter to finisher period. In these balance trials, no statistically significant difference has been observed in effects of the two phytases used Optiphos and Natuphos.

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