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Environmental aspects of the use of microbial phytase in the feed for pigs and poultry

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ABSTRACT

The influence of phytase supplementation in the feed on performance, nutrient digestibility and enzyme activity in the excreta was studied in experiments with growing pigs and laying hens. To a standard diet a commercial phytase was added according to the recommendations and in a ten-fold overdose. The experiments were performed with 15 growing pigs and 21 laying hens in individual pens and cages respectively. Although innate phytase activity in the pig diets was high phytase supplementation improved phosphorus digestibility. Performance and digestibility of energy and nitrogen were not affected significantly by the phytase supplementation. In laying hens phytase supplementation led only to a significant reduction of excreta dry matter content. In no case (pigs and poultry) a phytase activity could be detected in exreta.

Keywords: pig, laying hens, phytase, feedstuffs, environment

INTRODUCTION

The spare use of phosphorus (P) in animal nutrition is essential for an ecologically balanced agriculture. The nutrition of farm animals is focused on a low phosphorus supply that can cover the requirements according to the maximal performance expected. Like that an unnecessary surplus of phosphorus that is a load for the environment can be avoided. For this concept knowledge of the bioavailability of phosphorus in the feed is on one hand a precondition. On the other hand concepts are necessary to improve the availability especially of P from vegetable origin. In practice the use of enzymes like phytases to release P from phytate is a standard in regions with a delicate phosphorus balance (e.g. high animal density and open water flow). The effect of phytase to improve the digestibility of P can usually also be seen for other essential minerals and trace elements form vegetable feedstuffs (Jongbloed et al., 2004; Kies et al., 2005; Gebert et al., 1999). Of special interest is the availability of iron, zinc and copper.

The total phosphorus content in rations for pigs and poultry can be reduced by 1 to 1.5 g/kg if a phytase supplement of 500 FTU is added to the diet (Jongbloed et al., 2000; Johansen and Poulsen, 2003). The corresponding phosphorus amount will under these conditions be not excreted in the feces or in urine if the corresponding amount of P is reduced in the ration. This observation holds true for all other minerals that cannot be stored in animal tissues. From the large number of experiments with phytase supplementation it can furthermore be concluded, that beside a better mineral availability also an improved performance can be expected (Jongbloed et al., 2000).

It can not be excluded that the use of a phytase in animal feeds will have a detrimental environmental effect (Smith and Moore, 2005; Smith et al., 2005). If phytase is excreted in the feces there is the potential possibility of liberation of phosphorus in the soil and therefore an increase of phosphorus solubility and pollution in ground water can be expected. This aspect was therefore the background of two metabolism experiments with growing pigs and laying hens. The influence of phytase supplementation in the feed on performance, nutrient digestibility and enzyme activity in the excreta was studied. To a standard diet a commercial phytase was added according to the recommendations and in a ten fold overdose.

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MATERIAL AND METHODS

Pigs

The digestion experiments were performed with 15 growing pigs with a mean initial body weight (BW) of 23 kg and a final BW of approximately 104 kg. A cereal based standard diet A (Table 1) was calculated to contain 13.5 MJ digestible energy (DE), 170 crude protein (CP) and 4.46 g phosphorus (P) per kg in the growing, and 13.6 MJ DE, 150 g CP and 3.72 g P in the fattening period (above 60 kg BW) and to cover the requirement of all essential nutrients according to NRC (1998) . In treatment B a microbial phytase (Natuphos; BASF) was added in the practical dose of 500 FTU per kg. In treatment C a tenfold overdose of the phytase was applied (5000 FTU per kg). The diets were pelleted (4.5 mm diameter) at 60 °C. BW was recorded weekly. The animals were kept in individual pens and fed once daily according to a BW-based feeding scale (190 g x BW0.569) with water available ad libitum. Digestion experiments were performed according to the indicator method with HCl insoluble ash as indicator (Prabucki et al., 1975). For this purpose fresh representative feces samples were collected on 4 consecutive days at a mean body weight of 37.4 and 55.9 kg respectively.

Poultry

The experiment was performed with 21 laying hens (28 weeks old; 2.05 kg BW) randomly assigned to three feeding treatments X, Y and Z. The birds were kept in single cages, each equipped with a feeding trough, two nipple drinkers, a lying nest and a dust bath. The stable was air-conditioned and the light period amounted to 14 hours. A standard feed (X) mainly consisting of maize (40.5%), wheat (21.5%) and soybean meal (15%) (Table 1) with a calculated content of 11.6 MJ metabolizable energy (ME), 161 g crude protein and 4.68 g phosphorus per kg was supplemented with 300 FTU (Y) and 3000 FTU (Z) phytase (Natuphos) per kg feed, respectively. The diets were offered in mash form ad libitum. Performance data (feed intake, laying performance and egg weight) were recorded during 56 days (two laying periods of 28 days each). In each period representative excreta samples were collected over 4 days for the evaluation of energy utilization (indicator method) and phytase activity.

Analytical methods

Excreta samples of pigs and laying hens were freeze dried (Beta 116, Christ, Osterode am Harz, Germany). After 24-h air equilibration, samples were ground with a centrifugal mill (Retsch ZM 1) using a

Table 1: Composition of the pig and poultry basal diets A and X respectively (%)

	pig diet (A)		poultry diet (X)
	growing period	fattening period	
Maize			40.5
Barley	35	35	
Wheat	40	46.5	21.5
Extr. rapeseed meal	5	5	
Extr. soybean meal (43 % CP)	8	2	15
Dehydrated grass meal			5
Potato protein	2.5	2.5	3
Animal fat and molasses	5	5	
Soybean oil			3
Lysine-HCl	0.45	0.45	0.06
Methionine	0.09	0.05	0.22
Threonine	0.11	0.11	
Minerals	1.85	1.39	10.05
Vitamin-trace element premix	0.5	0.5	0.5
Propionic acid	0.1	0.1	
Celite 545[1]	1.4	1.4	1.17

[1]acid-washed diatomaceous earth

titanium sieve (0.5 mm) for subsequent analyses. Dry matter and crude nutrients in feed and excreta were determined according to the standard procedures of VDLUFA (Naumann et al., 1997). Gross energy was measured using an anisothermic bomb calorimeter (C 700 T, IKA Analysentechnik GmbH, Heitersheim, Germany). Acid insoluble ash (internal marker) content of feed and excreta was determined according to Prabucki et al. (1975). Phytase activity was analyzed at the Swiss Federal Station for Animal Production and Dairy Products (ALP), CH-Posieux according the VDLUFA-Methodenbuch III, chapter 27.1.1 (Naumann et al., 1997) and the Natuphos reference method (BASF).

The utilization of nutrients and energy was estimated by means of the indicator method (Prabucki et al., 1975; Vogtmann et al., 1975), using 4 N-HCl insoluble ash (AIA) as an inert indicator. Celite 545 (acid-washed diatomaceous earth), a nutritionally inert substance, was added to the diets to increase the level of AIA and reduce the variability in the analyses.

Statistical analysis

Experimental data were analyzed statistically by using SYSTAT 10.0 for Windows (2002) and treated by one-way ANOVA. To identify significant differences in pairs of means the Bonferroni Post hoc Test was used

Table 2: Nutrient and energy content as well as phytase activity in the diets

Treatment		growing pigs			fattening pigs			Laying hens		
		A	B	C	A	B	C	X	Y	Z
Phytase	FTU/kg	-	500	5000	-	500	5000	-	300	3000
Per kg in 88 % dry matter										
Crude ash	g		54			52			127	
Crude protein	g		170			140			162	
Crude fat	g		40			36			54	
Crude fiber	g		40			35			39	
Gross energy	MJ		16.1			16.5			15.3	
Phosphorus	g	5.26	5.33	5.17	4.40	4.35	4.34	5.07	4.73	4.97
Phytase activity[1]	FTU	600	1100	5980	710	1120	5760	172	614	3809

[1]Analysis according to the method Natuphos (BASF)

Table 3: Growth performance of the pigs (mean values and standard deviation)

Phytase	FTU/kg	Treatment A	Treatment B	Treatment C	p-value
		-	500	5000	
Body weight	kg				
begin		22.9 (0.60)	22.9 (0.96)	22.8 (0.19)	0.969
middle		59.8 (2.50)	61.4 (2.18)	61.4 (1.66)	0.857
end		103.1 (3.10)	106.2 (4.26)	102.9 (5.98)	0.508
Daily feed intake	kg				
growing period		1.53 (0.02)	1.54 (0.01)	1.53 (0.04)	0.456
f attening period		2.26 (0.07)	2.28 (0.04)	2.26 (0.03)	0.838
Daily body weight gain	g				
growing period		657 (59)	688 (57)	671 (80)	0.761
fattening period		823 (113)	855 (64)	790 (77)	0.519
Feed conversion ratio	kg/kg				
growing period		2.35 (0.23)	2.26 (0.20)	2.30 (0.27)	0.841
fattening period		2.78 (0.32)	2.67 (0.14)	2.88 (0.26)	0.439

at a significance level of P< 0.05. The individual pig or laying hen values were considered as experimental unit for the parameters examined.

RESULTS AND DISCUSSION

The analyzed nutrient and energy content of the pig and poultry diets (Table 2) corresponded well with the expected values. For phosphorus slightly higher values were observed. There was a big difference in phytase activity between the two diets without phytase supplementation (A and X). In diet A for pigs a high native phytase activity of 600 FTU per kg was observed probably due to the high percentage of wheat and barley which are known to contain a certain amount of innate phytase. Furthermore diets were pelleted at a low temperature (≈ 60°C) in order to protect the supplemented phytase as well as possible. By contrast in diet X for laying hens with only half as much wheat and no barley the corresponding activity amounted to only 172 FTU per kg. The added phytase in the other treatments corresponded well with the expected values. This demonstrates that the supplemented phytase and the innate as well resisted the heat load during the pelleting process in the pig diets.

The experiments with growing pigs between 23 and 106 kg body weight resulted in all treatments in similar performance data (see Table 3).

Daily body weight gain amounted to 657 and 823 g in the growing and fattening period respectively (control treatment A). With the phytase supplementation (500 FTU kg) in treatment B a slight increase of about 4 % occurred in the growing period. Almost the same tendency could be observed in the fattening period. Due to the small number of animals per treatment (n=5) this effect was not significant. The tenfold overdosing did not lead to a further increase. There was even a slight numerical decrease. Feed conversion ratio was also slightly improved with the phytase supplementation in treatment B, but not in treatment C with the tenfold overdosing.

In Table 4 the influence of the phytase supplementation on the digestibility of phosphorus, nitrogen and energy is listed in the four periods (I-IV) at 37.4, 55.9, 79.7 and 93.1 kg BW respectively. Furthermore, phytase activity in the feces is presented. Mean phosphorus digestibility decreased in treatment A from 0.50 to 0.44 over the four collection periods. In this treatment native phytase activity amounted already to 600 FTU per kg. The further supplementation of 500 FTU per kg in treatment B had as a consequence an increase of phosphorus digestibility of 23 %. Only a minor further increase of about 4 % was observed with the tenfold supplementation with the phytase. Nitrogen and energy digestibility were very homogenous over all collection periods from 37 to 93 kg BW. Furthermore no significant treatment effects with the phytase supplementation on

Table 4: Nutrient and energy digestibility in growing pigs and phytase activity in the feces (mean values and standard deviation)

		Treatment			p-value
		A	B	C	
		-	500	5000	
Phytase Digestibility	FTU/kg				
Phosphorus	I	0.501 (0.050)	0.600 (0.027)	0.581 (0.134)	0.182
	II	0.426 a(0.048)	0.536 b(0.039)	0.555 b(0.066)	0.004
	III	0.438 a(0.039)	0.563 b(0.057)	0.596 b(0.063)	0.002
	IV	0.454 a(0.042)	0.542 b(0.049)	0.592 b(0.056)	0.003
Nitrogen	I	0.860 (0.011)	0.869 (0.017)	0.855 (0.013)	0.298
	II	0.865 (0.010)	0.874 (0.014)	0.862 (0.005)	0.278
	III	0.891 (0.008)	0.881 (0.008)	0.881 (0.008)	0.129
	IV	0.880 (0.008)	0.888 (0.003)	0.879 (0.006)	0.055
Energy	I	0.853 (0.008)	0.852 (0.012)	0.844 (0.005)	0.194
	II	0.864 (0.010)	0.861 (0.009)	0.851 (0.006)	0.066
	III	0.865 (0.009)	0.856 (0.008)	0.854 (0.008)	0.139
	IV	0.857 (0.010)	0.862 (0.004)	0.857 (0.005)	0.447
Phytase activity FTU/kg[1)] (I,II)		<100 [2)]	<100 [2)]	<100 [2)]	

[ab]within a row means without a common superscript letter differ significantly (p < 0.05)
I-IV: determined at 37.4, 55.9, 79.7 and 93.1 kg BW, respectively; [1)] in feces according to the method Natuphos (BASF); [2)] below detection limit

nitrogen and energy digestibility were observed although the digestibility values were highly uniform and some increase of performance could be derived. In no single feces sample any phytase activity could be measured. It must be considered that in treatment A an innate phytase activity of 600 FTU per kg existed. Under this precondition our results are in good agreement with corresponding literature (Jongbloed et al., 2000; Traylor et al., 2001; Kies et al., 2005).

As already observed in the experiments with pigs phytase supplementation did not influence the performance of laying hens significantly (Table 5) although phytase activity in treatment X amounted only to 172 FTU per kg. In treatments Y and Z a marginal reduction of feed intake was observed in comparison with control diet X. This effect could not be explained by the phytase supplementation. The same observation was also made with the hens' laying performance as well as egg weight. Liebert et al. (2005) found similar results. In their experiments only feed conversion ratio was improved with phytase supplementation in low phosphorus diets. Phytase supplementation had almost no effect on metabolizability of energy. Because the interpretation of availability of phosphorus in laying hens without special investigations is not possible the results are not discussed here (Rodehutscord et al., 2002). In treatment Y and Z phytase supplementation led to a reduction of dry matter content in excreta without causing any health consequences. Likewise to the pig experiments no phytase activity could be detected in the poultry excreta. It can therefore be concluded from both experiments (pigs and laying hens) that the enzymatic activity of phytase was completely inactivated by the digestion process.

Today it is of central interest to feed pigs and poultry according to their nutrient and energy requirements. This concept includes beside the nutrient content of the diet and the daily feed intake also the availability of the nutrients. The use of exogenous enzymes and especially of phytases can substantially increase the digestibility and absorption rate of phosphorus as well as other minerals and trace elements. In some cases even the digestibility of protein (amino acids) and energy are better utilized with the use of phytases. The concept of feeding available nutrients to farm animals includes the reduction of the content of essential nutrients to some degree compared to the former crude nutrient concept if the nutrient availability is known and as high as possible. This is of great importance for environmental aspects because unutilized nutrients are excreted as feces or urine. The use of phytases in animal nutrition is therefore then meaningful when the improved nutrient availability is compensated by a reduced nutrient content in the diet.

The main environmentally beneficial effect of the phytase supplementation of pig and poultry diets is based on the reduced excreted nutrient load per animal (Maguire et al., 2004; McGrath et al., 2005). There is also a reduced amount of phytate in excreta. This represents only a minor contribution since plants are able to produce and excrete phytases in their roots to mineralize organic phosphorus (Tarafdar et al., 2005). Furthermore diverse soil microorganisms can contribute to the phosphorus turnover with the production of phytases as well (Chadha et al., 2004; Zinin et al., 2003; Yadav and Tarafdar, 2003). A further effect of feed enzymes can therefore be excluded since these enzymes are broken down in the digestive tract and are not excreted.

Table 5: Performance and metabolism data as well as excreta phytase activity of the laying hens (mean values and standard deviation)

			Treatment		
			X	Y	Z
Phytase	FTU/kg		-	300	3000
Body weight	g	begin	2045 (93)	2061 (121)	2046 (81)
		end	2082 (79)	2113 (145)	2041 (94)
Feed intake	g/d		114.6 (3.6)	112.3 (5.9)	111.8 (9.4)
Laying performance	%		98.1 (2.8)	96.1 (3.9)	97.3 (2.4)
Egg weight	g		67.8 (6.9)	65.6 (5.0)	64.8 (3.5)
Feed per kg egg mass	kg		1.737(0.168)	1.792 (0.165)	1.773 (0.121)
m(E) 3)			0.755 (0.037)	0.760 (0.021)	0.744 (0.028)
Excreta dry matter	g/kg 3)		236a (34)	215ab (28)	201b (19)
Phytase activity 1)	FTU/kg		<100 2)	<100 2)	<100 2)

ab within a row means without a common superscript letter differ significantly ($p < 0.05$)
1) in excreta according to the method Natuphos (BASF) 2) below detection limit 3) data of both collecting periods

CONCLUSIONS

The missing effect of the phytase supplementation in diets for pigs and poultry on the phytase activitiy in the excreta in both animal species in our experiments demonstrate, that exogenous phytase is completely inactivated during digestion processes like any other protein source. It can be therefore concluded that such excrements as fertilizer will not increase the solubilization of phosphorus in the soil. Even a tenfold overdose did not show any influence.

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