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Dietary effect of phytogenic phytase and an addition of microbial phytase to a diet based on field beans, wheat, peas and barley on the utilization of phosphorus, calcium, magnesium, zinc and protein in piglets*

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Effekt pflanzlicher Phytase und mikrobieller Phytasezulagen zu einer Diät aus Ackerbohnen, Weizen, Erbsen und Gerste auf die Verwertung von Phosphor, Calcium, Magnesium, Zink und Protein bei Ferkeln

Summary: The effect of the addition of microbial phytase to a diet based on field beans (30 %), wheat (28 %), peas (25 %), and barley (14 %) was studied in a 2-week experiment with 3 x 8 castrated male, individually housed, hybrid piglets (live weight range 12–16 kg). All diets contained about 4.7 g Ca, 4.2 g P (77 % present as phytate phosphorus), 1.0 g Mg, 60 mg Zn per kg diet, and 17 % crude protein. Group I was fed the basal diet with a native phytase-activity of about 260 U per kg diet. In group II, 350 U, in group III, 700 U of microbial phytase per kg diet were added. The addition of microbial phytase improved the apparent P absorption (% of intake) from 48 % (group I) to 66 % (group II) and 71 % (group III). Comparable positive effects from the phytase treatment were obtained for the calcium utilization. The phytase supplementation also enhanced plasma zinc concentration significantly. The concentration of inorganic phosphorus in plasma, the zinc digestibility, and the magnesium balance were improved in tendency. The utilization of nitrogen remained unchanged.

Zusammenfassung: In einem zweiwöchigen Stoffwechselversuch mit 3 x 8 männlichen kastrierten Hybridferkeln (Lebendmassebereich 12–16 kg) wurde die Wirkung einer Zulage an mikrobieller Phytase zu einer Diät auf der Basis von Ackerbohnen (30 %), Weizen (28 %), Futtererbsen (25 %) und Gerste (14 %) untersucht. Die Diäten enthielten je kg Diät ca. 4,7 g Ca, 4,2 g P (davon ca. 77 % Phytin-P), 1,0 g Mg, 60 mg Zn sowie 17 % Rohprotein. Zum nativen Phytasegehalt von ca. 260 U/kg Diät (Gruppe I) wurden in den Gruppen II und III 350 bzw. 700 U mikrobieller Phytase/kg Diät supplementiert. Durch die Zulage von 350 und 700 U mikrobieller Phytase je kg Diät wurde die scheinbare P-Absorption (in % der Aufnahme) von 48 % in der Kontrollgruppe (Gruppe I) auf 66 % (Gruppe II) und 71 % (Gruppe III) gesteigert. Ähnlich positive Effekte traten bei der Ca-Verwertung auf. Auch die Plasma-Zn-Konzentration wurde durch die Phytasesupplementierung signifikant gesteigert. Die Gehalte an anorganischem

Abbreviation Index:

AAS	Atomic absorption spectrometry	SD	Standard deviation
HPLC	High performance liquid chromatography	SEM	Standard error of the mean
PA	Phytic acid	XP	Crude protein

* Dedicated to Prof. Dr. agr. Dr. med. vet. h.c. Dr. agr. h.c. mult. M. Kirchgeßner on the occasion of his 65. birthday

Phosphat des Blutplasmas, die scheinbare Zn-Absorption und die Magnesiumverwertung wurden durch die Phytasezulage in der Tendenz erhöht, während die N-Bilanz unbeeinflußt blieb.

Key words: Phytate – microbial phytase – mineral utilization – protein – pigs

Schlüsselwörter: Phytat – mikrobielle Phytase – Mineralstoffverwertung – Protein – Schwein

Introduction

About two-thirds of the phosphorus in cereals, legumes, and oilseeds are present as phytate-phosphorus (11, 13). Since man and monogastric animals have no or little intestinal phytase (12, 27, 31, 37), phytate-phosphorus is less available to nonruminants.

Successful attempts have been made to produce high levels of extracellular microbial phytase. Compared to phytase of plant origin, fungal *Aspergillus*-phytase is stable over a wide pH-range with two distinct pH-optima at pH 2.5 and 5.0–5.5 (5, 34, 36, 38). Recent studies have shown that phytase-supplementation to phytate-rich diets enhances phosphorus utilization and bone mineralization in pigs (10, 14, 15, 18, 24, 26, 34) and chickens (33, 34, 35).

Phytic acid (PA) also interferes with essential divalent cations such as calcium (6, 14, 16, 24), magnesium (2, 14, 25), and zinc (4, 9, 16, 17, 22, 23, 29, 30) by forming insoluble complexes under the pH conditions of the small intestine. Negative effects of phytate upon protein digestibility in vitro (3) and in vivo (1) have also been described. In most previous studies the effects of microbial phytase on phosphorus utilization were studied in maize-soya diets, which are very low in native phytase activity. The objective of the present study was to elucidate whether an addition of microbial phytase to a diet based on field beans, wheat, peas, and barley improves the utilization of phosphorus, calcium, magnesium, zinc, and nitrogen in growing pigs.

Materials and methods

3 x 8 castrated male, hybrid piglets (live weight range = 12–16 kg) were used. The animals were kept at 26 °C and 60 % humidity, individually in metabolic cages which allowed complete and separate collection of urine and feces. For 2 weeks the piglets were fed diets on the basis of field beans (*Vicia faba*), wheat, peas (*Pisum sativum*), and barley (Table 1). Group I was fed the basal diet (containing 0.42 % native phosphorus) without an addition of microbial phytase. In group II, 350 U, in group III, 700 U microbial phytase per kg diet from *Aspergillus niger var. van tighem* (BASF AG, Ludwigshafen) were added. The diets (600 g per day) were offered twice daily in pelleted form. The animals had free access to bidistilled water.

To evaluate apparent absorption of zinc and apparent absorption and retention of phosphorus, calcium, magnesium, and nitrogen, a 7-day balance trial was performed at the end of the experiment (days 7–14). Feces and urine were collected daily. Blood samples were taken at the end of the experiment (day 14) by puncturing the *vena jugularis*. Dietary PA was analyzed by HPLC (20). Activity of phytase was assayed according to the method of Fretzdorff and Weipert (7). One unit (U) phytase is defined as the amount of enzyme required to liberate 1 µmol inorganic phosphorus from sodium phytate (1.5 mmol) in 1 min at 37 °C and pH 5.5 (33). Calcium, magnesium, and zinc in diets, freeze-dried feces, and urine were determined by flame atomic absorption spectrophotometry (Philips PU 9485). Total phosphorus was determined colorimetrically by the vanadomolybdate procedure (19). Samples were dried at 105 °C in quartz glass cru-

Table 1. Composition of the basal diet

Ingredients	(g/kg)
Field beans	300.0
Wheat	277.1
Peas	250.0
Barley	140.0
Wheat bran	10.0
$CaCO_3$	12.5
NaCl	3.0
DL-Methionine	2.4
L-Lysine-HCl	1.0
Choline chloride	2.0
Vitamin mixture*	1.0
Trace element mixture**	1.0
	1000.0

* Vitamin mixture:
Amount supplied (per kg diet): 10 000 I.U. vitamin A, 1000 I.U. vitamin D_3, 30 I.U. vitamin E, 3 mg vitamin K_3, 100 mg ascorbic acid, 3 mg thiamin, 4 mg riboflavin, 4 mg pyridoxine, 30 µg vitamin B_{12}, 0.3 mg biotin, 2 mg folic acid, 40 mg nicotinic acid, 30 mg pantothenic acid, 3 mg inosite

** Trace element mixture:
Amount supplied (per kg diet): 30 mg Zn as $ZnSO_4$ x 7 H_2O, 30 mg Fe as $FeSO_4$ x 7 H_2O, 5 mg Cu as $CuSO_4$ x 5 H_2O, 0.3 mg Se as Na_2Se0_3 x 5 H_2O, 0.2 mg I as KI

cibles, dry ashed at 450 °C and dissolved in 0.3 M HCl (suprapure for the AAS). Plasma was diluted with 0.3 M HCl prior to zinc analysis. Nitrogen was analyzed by the Kjeldahl method (19). Crude protein (XP) was calculated as Kjeldahl-N x 6.25. Inorganic phosphorus in plasma was assayed by a test-kit (Boehringer Mannheim Nr. 124971).

Standard ANOVA procedure (oneway) followed by Scheffé multiple-range-test was used for statistical analysis. Differences were considered significant if $p < 0.05$.

Results

As shown in Table 2, the three diets for the piglets contained 4.6–4.8 g calcium, 1.0 g magnesium (native concentration), and about 60 mg zinc (30 mg native plus 30 mg as

Table 2. Analyzed composition of experimental diets

	Diet		
	I	II	III
Ca (g/kg)	4.6	4.7	4.8
P (g/kg)	4.2	4.3	4.2
Mg (g/kg)	1.0	1.0	1.0
Zn (mg/kg)	57	60	60
XP (g/kg)	172	174	176
Phytate (g/kg)	11.3	11.8	11.8
Phytase (U/kg)	258	595	840

$ZnSO_4$ x 7 H_2O) per kg diet (dry matter 85.5 %). In all diets 4.2–4.3 g total phosphorus per kg diet were analyzed. The concentration of phytic acid ranged between 11.3–11.8 g per kg diet, thus about 77 % of the total phosphorus in the diets were present as phytate-phosphorus. In group I a native phytase-activity of about 260 U/kg diet was measured. The analyzed phytase-activity in group III was lower than expected as only 85 % of the added microbial activity were recovered. Possibly some enzyme-activity was lost during the pelleting process.

The effect of microbial phytase on the apparent absorption and retention of phosphorus, calcium, and magnesium is given in table 3. In group I (control) the lowest apparent P absorption (48 % of intake) was measured. The addition of 350 and 700 U microbial phytase per kg diet significantly increased apparent P absorption to 66 % (group II) and 71 % (group III). Daily urinary P excretion in group I was negligible (0.01 g/d), while in groups II (0.09 g/d) and III (0.20 g/d) much higher amounts of phosphorus were excreted via urine. Differences in P utilization were also reflected by the plasma-concentration of inorganic phosphorus. In comparison to group I (control), pigs fed diets supplemented with microbial phytase exhibited in tendency ($p < 0.1$) higher concentrations of inorganic phosphorus in plasma. The following mean values (± SD) for the plasma-concentration of inorganic phosphorus (mmol/L) were measured: 2.40 ± 0.34 (group I), 2.60 ± 0.29 (group II) and 2.58 ± 0.25 (group III). Under the conditions investigated, calcium digestibility was improved by phytase supplementation and retained Ca was significantly higher than from the control diet (Table 3). Daily urinary Ca excretion in piglets fed the basal diet was 2–3 fold higher than that of those fed the diets supplemented with microbial phytase. The addition of microbial phytase also had a significantly positive effect on the amount of Mg retained. The apparent absorption of Mg was increased in tendency ($p < 0.1$) by the phytase treatment (Table 3).

All diets contained 57–60 mg Zn per kg diet and 1.1–1.2 % PA, thus molar PA : Zn-ratios of 20 : 1 were obtained. The apparent Zn absorption tended ($p < 0.1$) to be higher in the phytase groups (Fig. 1). Also shown in Fig. 1 are significant differences in the zinc status of the animals, demonstrated by the plasma zinc concentration.

The diets contained about 17 % crude protein. For apparent N absorption (% of intake) 85.5 ± 3.2 (group I), 85.2 ± 3.7 (group II), 84.3 ± 1.5 (group III) and for apparent N retention (% of intake) 58.8 ± 6.5 (group I), 54.0 ± 5.6 (group II), 55.3 ± 3.8 (group III) were measured. At the crude protein dietary level investigated there was no significant difference in N absorption and N balance among the three groups.

Discussion

Compared to the control group, phytase supplementation resulted in a decrease of daily fecal P excretion of 39 % (group II) and 49 % (group III). This is similar to results reported for pigs fed maize-soya diets supplemented with microbial phytase (24, 34). On the assumption that 80 % of the non phytic acid P is digestible, it can be calculated that in group I (control) about 37 % (1.2 g phytate P per kg diet) of the phytate P were absorbed. In contrast to the present study with a diet of field beans, wheat, peas and barley, previous experiments with a maize-soya diet containing no or little native phytase of plant origin showed that phytate P was indigestible. These differences found in P absorption therefore seem to be related to differences in the native phytase content of the diets. By the addition of 350 and 700 U microbial phytase per kg diet, the calculated digestibility of phytate P increased to 60 % (group II) and 69 % (group III).

Table 3. Effect of microbial phytase supplementation to a diet based on field beans, wheat, peas, and barley on apparent digestibility and balance of phosphorus, calcium and magnesium in piglets (n = 3 x 8)

	Phosphorus				Calcium				Magnesium			
Group	I	II	III	SEM*	I	II	III	SEM	I	II	III	SEM
Intake, g/d	2.66[a]	2.46[a]	2.51[a]	0.0690	2.96[a]	2.65[a]	2.87[a]	0.0779	0.633[a]	0.588[a]	0.630[a]	0.0165
Absorbed, g/d	1.27[a]	1.61[b]	1.79[b]	0.0621	1.75[a]	1.78[a]	2.08[a]	0.0601	0.223[a]	0.248[a]	0.253[a]	0.0071
Apparent absorption, % of intake	47.9[a]	66.5[b]	71.2[b]	2.38	59.1[a]	67.9[b]	72.3[b]	1.54	35.5[a]	44.7[a]	40.6[a]	2.05
Urinary, g/d	0.01[a]	0.09[b]	0.20[c]	0.0199	0.15[a]	0.07[ab]	0.05[b]	0.0154	0.065[a]	0.038[a]	0.047[a]	0.0059
Retained, g/d	1.27[a]	1.53[ab]	1.56[b]	0.0508	1.60[a]	1.71[ab]	2.01[b]	0.0641	0.156[a]	0.211[b]	0.211[ab]	0.0099
Apparent retention, % of intake	47.7[a]	62.8[b]	62.8[b]	1.90	54.4[a]	65.3[ab]	70.6[b]	2.43	24.9[a]	38.5[a]	34.3[a]	2.45

Different letters within the same row show significant differences ($p < 0.05$) between the groups

* Standard error of the mean

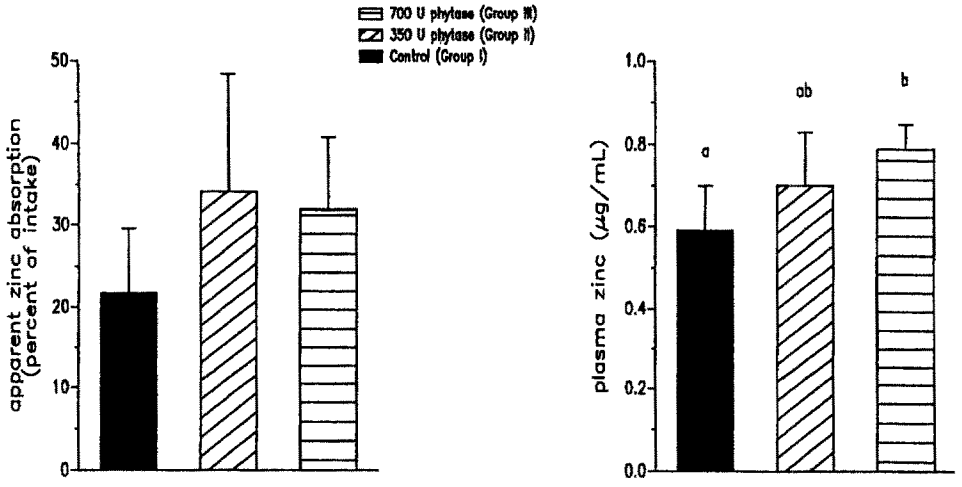

Fig. 1. Effect of microbial phytase supplementation to a diet based on field beans, wheat, peas, and barley on apparent zinc absorption (% of intake) and plasma zinc concentration (µg/mL) in piglets. Different letters show significant differences ($p < 0.05$) between the groups.

The supplementation of microbial phytase also increased the Ca utilization significantly. This is in accordance with results for pigs and chickens (14, 24, 34, 35). The low apparent absorption and retention of calcium in group I (control) might be explained by an insufficient phytate hydrolysis in the stomach followed by the formation of largely insoluble calcium phytate complexes under the pH conditions of the small intestine.

In group I (control) renal P excretion (0.01 g/d) was practically negligible, while a high renal Ca excretion (0.15 g/d) was observed. Hypophosphaturia and hypercalciuria are described as typical symptoms of P deficiency (26, 28) and are assumed to be related to the insufficient bioavailability of phytate phosphorus in the basal diet.

In studies with rats it has been shown that phytate inhibits the Mg absorption (2). In the present experiment Mg utilization tended to be higher with the phytase treatment. This is in agreement with previous findings for pigs (14, 25) when diets based on maize and soya were fed.

An inverse relationship between dietary phytate and zinc utilization in rats (4, 6, 16, 17, 23, 29, 30), chickens (22, 35), pigs (14, 15, 25), and humans (32) has also been reported. In particular molar PA : Zn-ratios > 15–20 : 1 induce suboptimal zinc status in rats (4, 6, 17). In the present trial diets with PA : Zn-ratios of 20 : 1 were fed. The addition of 350 and 700 U microbial phytase per kg diet resulted in a moderate improvement in zinc digestibility and plasma zinc concentration. In an earlier study with growing rats more substantial effects of a supplementation of *Aspergillus*-phytase on the zinc utilization were observed when phytate-rich diets containing only 16–20 ppm Zn and high levels of Ca (0.80 %) were fed (29, 30). Compared to the recommended requirement (8, 21), dietary levels of calcium (0.46–0.48 %) in this study were low and thus differences in the zinc utilization might also be related to differences in the calcium levels of the diets.

Phytic acid is known to form complexes also with proteins under the pH conditions of the gastrointestinal tract and thus stepwise hydrolysis of phytate protein complexes may increase the digestibility of protein and bioavailability of essential amino acids. In the

present study there was no effect of phytase supplementation on N utilization. This is in agreement with the findings of Näsi (18). Diets were calculated to provide sufficient amounts of essential amino acids and crude protein. More nutritional experiments are however necessary to study interactions between phytate, microbial phytase, and protein utilization under marginal or suboptimal dietary conditions.

In conclusion, it could be shown that supplementing phytate-rich diets based on field beans, wheat, peas, and barley with microbial phytase improved bioavailability of phytate phosphorus as well as the utilization of calcium in growing pigs. In addition, a moderate increase of the utilization of magnesium and zinc was observed by the enzyme supplementation.

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