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Enhancement of zinc utilization from phytate-rich soy protein isolate by microbial phytase

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Verbesserung der Zinkverwertung aus phytatreichem Sojaproteinisolat durch mikrobielle Phytase

Summary: A study with three groups, each with 11 male, individually housed albino rats (initial average weight = 50 g) was undertaken to examine the effect of microbial phytase (added to a diet containing phytate) on the availability of zinc. The rats were fed diets on the basis of soy protein isolate and corn starch over a 3-week period. All diets contained 15–16 mg Zn/kg diet and 0.40 % PA. Thus, molar PA : Zn-ratios of 26 : 1 were obtained. Group I (control) was fed the phytase-free basal diet. In groups II (pair-fed to group I) and III, 1 000 U of microbial phytase (*Aspergillus niger var. van tighem*) per kg diet were added.

Some rats fed the phytase-free basal diet (control) showed typical symptoms of zinc deficiency, including cyclic changes in food intake, anorexia and partial alopecia.

By the addition of 1 000 U microbial phytase the apparent absorption of zinc (percent of intake) significantly increased from 33 % (control) to 63 % (1 000 U, pair-fed) and 66 % (1 000 U, ad lib.). Similar positive effects of the phytase-supplementation were observed for three zinc status parameters in plasma, zinc-concentration, percent unsaturated zinc-binding capacity, activity of alkaline phosphatase and the zinc-concentration in femur and testes. The present study shows that an addition of microbial phytase to phytate-rich diets based on soy protein isolate considerably improves the availability of zinc in growing rats.

Zusammenfassung: In einem 3wöchigen Stoffwechselexperiment mit 3 x 11 männlichen, einzeln gehaltenen Albinoratten (Anfangsmasse = 50 g) wurde geprüft, ob eine Zulage an mikrobieller Phytase die Bioverfügbarkeit von Zink aus einer phytathaltigen Diät verbessert. Den Ratten wurden Diäten auf der Basis von Sojaproteinisolat und Maisstärke verabreicht. Der native Zn-Gehalt aller Diäten betrug 15–16 mg Zn/kg Diät, die PA-Konzentration 0,40 %. Daraus resultierten molare PA : Zn-Quotienten von 26 : 1. Gruppe I (Kontrolle) erhielt die phytasefreie Basisdiät. In den Gruppen II (pair-fed zu I) und III (ad libitum) wurden jeweils 1 000 U mikrobieller Phytase (*Aspergillus niger var. van tighem*) pro kg Diät supplementiert.

Abbreviation Index:

AAS	Atomic absorption spectrometry	M	Mean
ALP	Alkaline phosphatase	PA	Phytic acid
DM	Dry matter	SD	Standard deviation
FCR	Feed conversion ratio	ZBC	unsaturated plasma-zinc binding capacity
HPLC	High performance liquid chromatography		

Einige Ratten der Kontrollgruppe, die die phytasefreie Basisdiät erhielten, zeigten typische Zinkmangelsymptome wie Anorexie, partielle Alopezie und zyklische Schwankungen der Futteraufnahme. Durch die Phytasezulage wurde die scheinbare Absorption des Zinks (in % der Aufnahme) signifikant auf 63 % (1 000 U, pair-fed) und 66 % (1 000 U, ad lib.) gegenüber 33 % (phytasefreie Kontrollgruppe) erhöht. Ein vergleichbar positiver Effekt der Phytasesupplementierung wurde bezüglich verschiedener Zinkstatusparameter des Blutplasmas (Zinkkonzentration, freie Zinkbindungskapazität, Aktivität der Alkalischen Phosphatase) und der Zinkeinlagerung in Femur und Testes festgestellt. In der vorliegenden Untersuchung konnte gezeigt werden, daß bei der wachsenden Ratte durch eine Zulage mikrobieller Phytase die Bioverfügbarkeit des Zinks in phytatreichen Diäten auf Sojaproteinisolatbasis sehr deutlich gesteigert werden kann.

Key words: Phytate – soy protein isolate – microbial phytase – zinc-absorption – zinc-status

Schlüsselwörter: Phytat – Sojaproteinisolat – mikrobielle Phytase – Zink-Absorption – Zink-Status

Introduction

Soy products which contain considerable quantities of phytic acid (PA) play an important role in human and animal nutrition (5, 16, 35). The concentration of phytic acid in soy protein isolate ranges from 1.6–2.2 g/100 g DM (1, 4). Phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), the storage form of organic phosphorus in plant seeds and derived food and feedstuffs is generally considered to reduce the availability of numerous essential divalent cations through the formation of complexes which are largely insoluble for nonruminants. A number of investigators reported an inverse relationship between dietary phytate and zinc utilization in rats (2, 7, 9, 18), chickens (20), pigs (11) and humans (17, 27). High levels of dietary calcium aggravate the antinutritive effect of phytic acid on zinc availability. Molar PA : Zn-ratios > 15–20 : 1 (10, 18, 22, 25) and molar PA x Ca : Zn-ratios > 3.5 : 1 (6) induce suboptimal zinc status in rats. Since monogastric animals have no or little intestinal phytase, it might be advantageous to reduce phytic acid in phytate-rich diets in order to improve the availability of zinc and other essential minerals (4, 10, 24).

New techniques have been developed to produce high levels of an 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 5.5 (12, 29, 31). Recent studies showed that a phytase-supplementation to phytate-rich soy-maize-diets enhances the utilization of calcium and phosphorus in pigs (23, 29) and chickens (28, 30). The main objective of the present study was to elucidate whether an addition of microbial phytase to a diet based on soy protein isolate and corn starch improves the utilization of zinc in growing rats.

Methods

Male, weaned albino rats (Wistar, SPF) with an initial average weight of 50 g were divided into three groups of 11 rats and kept individually under standard conditions (22 °C, 55 % humidity, 12 h light/dark cycle) in metal free metabolic cages. For three weeks the rats were fed diets on the basis of soy protein isolate (Condimenta S 90, Stuttgart) and corn starch (Table 1).

Table 1. Composition of the basal diet

Ingredients	g/kg
Soy protein isolate	376
Corn starch	300
Saccharose	100
Soy oil	70
Cellulose	30
DL-methionine	4
Mineral mixture*)	100
Vitamin mixture**)	20
	1000

*) Mineral mixture (per kg diet)
13.34 g $CaHPO_4 \times 2\ H_2O$; 6.68 g KH_2PO_4; 5.07 g $MgSO_4 \times 7\ H_2O$; 12.24 g $CaCO_3$; 2.47 g NaCl; 1.22 g Na_2CO_3; 59.70 mg $FeSO_4 \times 7\ H_2O$; 140.83 mg $MnSO_4 \times H_2O$; 11.80 mg $CuSO_4 \times 5\ H_2O$; 4.41 mg $(CH_3COO)_3Cr$; 2.38 mg $CoSO_4 \times 7\ H_2O$; 2.21 mg NaF; 0.83 mg $Na_2SeO_3 \times 5\ H_2O$; 0.65 mg KJ; 0.25 mg $Na_2MoO_4 \times 2\ H_2O$

**) Vitamin mixture (per kg diet)
1.8 mg retinol; 0.025 mg cholecalciferol; 100 mg DL-α-tocopheryl acetate; 5 mg menadione; 30 mg ascorbic acid; 8 mg thiamine mononitrate; 10 mg riboflavin; 10 mg pyridoxine; 40 mg niacin; 30 mg Ca-D-pantothenat; 3 mg folic acid; 10 mg p-aminobenzoic acid; 0.2 mg biotin; 0.05 mg cobalamin; 100 mg myo- inositol; 1150 mg choline chloride

Group I (control) was fed the basal diet, free of microbial phytase. In group II (pair-fed to group I) and group III (ad libitum) 1 000 U of microbial phytase (*Aspergillus niger var. van tighem*, BASF AG, Ludwigshafen) were added.

The animals had free access to bidestilled water. Live weights were recorded weekly. During the second week (days 7–14) total feces and urine were collected.

Dietary PA was estimated by HPLC (19). Activity of phytase in the diets was assayed according to the method of Fretzdorff and Weipert (8). One unit (U) phytase is defined as the amount of enzyme required to liberate one μmol inorganic phosphorus from 1.5 mmol sodium phytate in 1 min at 37 °C and pH 5.5 (28). Zinc- and calcium-concentrations in diets as well as zinc-concentration in tissues, feces, urine and plasma were determined by flame atomic absorption spectrophotometry (Philips PU 9485) in an air-acetylene flame. Plasma was diluted with 0.3 M HCl prior to zinc analysis. Samples were dried at 105 °C in quartz dishes until weight constancy, dry-ashed at 450 °C, and dissolved in 0.3 M HCl (suprapure for the AAS).

The activity of the alkaline phosphatase (ALP) was assayed according to the guidelines of the Deutsche Gesellschaft Klinische Chemie (3). The method of Kincaid and Cronrath (14) in the modification of Roth and Kirchgeßner (26) was used after reduction of sample volume to estimate percent unsaturated plasma-zinc binding capacity (ZBC).

Standard ANOVA procedure (oneway) followed by Scheffé multiple-range test was used for statistical analysis. All values are expressed as means (M) and standard deviations (SD). Differences were considered significant if $p < 0.05$.

Results and discussion

As shown in Table 2 all diets contained 15–16 mg Zn (native Zn-concentration), 8.4–8.5 g Ca and 5.6–5.7 g P per kg diet and 0.40 % PA, and thus molar PA : Zn-ratios of 26 : 1 and molar PA x Ca : Zn-ratios of 5.3 : 1 were obtained. About 20 % of total phosphorus in the diets were present as phytate-phosphorus. The analytical recoveries of supplemented microbial phytase in groups II and III were adequate. In group I (control) no native phytase activity was detectable.

Table 2. Analyzed concentrations of Zn, Ca, PA as well as phytase activity and molar PA : Zn- and molar PA x Ca : Zn-ratios in the three diets for growing rats

Group	n	Feeding-regimen	Zn (mg/kg)	Ca (g/kg)	P (g/kg)	PA (%)	Phytase (U/kg)	PA : Zn (molar)	PA x Ca : Zn (molar)
I	11	ad libitum	15.5	8.5	5.7	0.40	–	26 : 1	5.3 : 1
II	11	pair-fed to I	15.2	8.5	5.6	0.40	1030	26 : 1	5.3 : 1
III	11	ad libitum	15.3	8.4	5.6	0.40	960	26 : 1	5.3 : 1

Some rats fed the phytase-free basal diet showed typical symptoms of zinc deficiency including cyclic changes in food intake, anorexia and partial alopecia as described in earlier studies (21, 22, 32, 33).

Fig. 1 shows growth curves of rats fed the soy protein isolate based diets with and without an addition of microbial phytase. At the start of the experiment, the 33 rats weighed 50.6 ± 1.9 g. At the completion of the experiment the average weights of the rats were 108.8 ± 12.0 g (control), 119.9 ± 3.7 g (1 000 U, pair-fed), 153.8 ± 11.7 g (1 000 U, ad lib.) with statistically significant differences between all three groups. By the addition of 1 000 U microbial phytase, the FCR was improved from 1 : 2.65 (control) to 1 : 2.17 (1 000 U, pair-fed) and 1 : 2.34 (1 000 U, ad lib.).

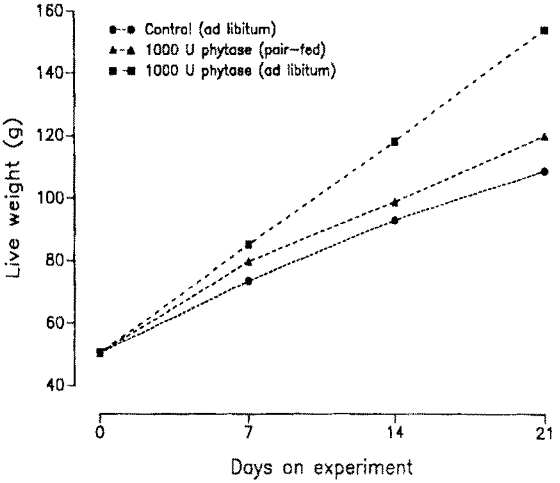

Fig. 1. Live weight gain of rats when microbial phytase is added to a phytate-rich diet based on soy protein isolate and corn starch.

The effects of the supplementation of microbial phytase on the apparent absorption and retention of zinc (percent of intake) are given in Fig. 2. In group I (control), the lowest apparent zinc-absorption (37.2 ± 5.3 %) was measured. The addition of 1000 U microbial phytase per kg diet resulted in a significant enhancement of the apparent absorption of zinc in groups II (62.9 ± 2.2 %) and III (66.4 ± 5.6 %). This is in agreement with an earlier study with growing rats (25) when semisynthetic diets based on egg albumin and corn starch supplemented with NaPA and *Aspergillus*-phytase were fed. Other investigators also found an increase in zinc-absorption by the addition of microbial phytase to phytate rich corn-soy diets in pigs (15, 24) and chickens (30). About 95 % of the total zinc-excretion was via feces and, thus, similar results for the apparent zinc-retention were obtained. The following average values for the apparent zinc-retention were measured: 34.7 ± 5.3 % (control), 60.0 ± 2.0 % (1000 U, pair-fed) and 64.9 ± 5.6 (1000 U, ad lib.). The renal zinc-excretion ranged from 3.6–4.0 μg Zn per animal and day and was not significantly influenced by the different treatments.

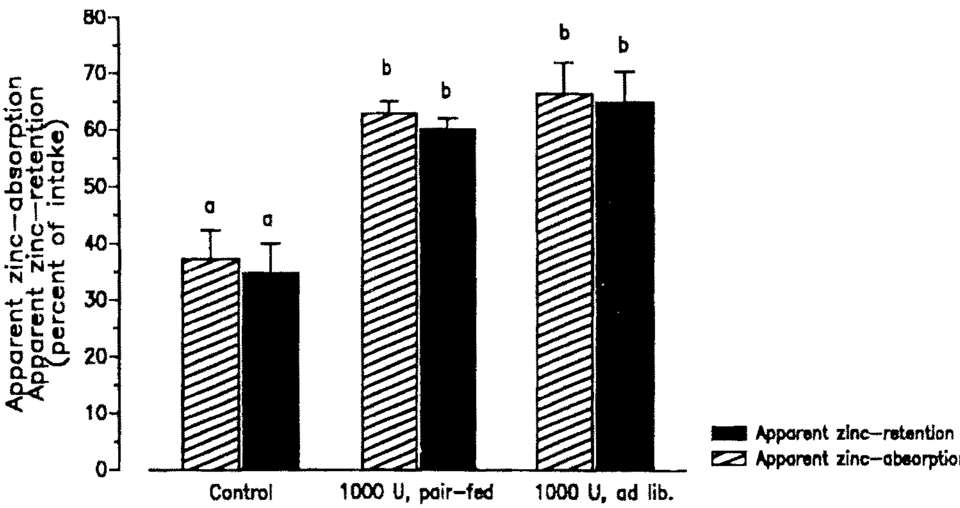

Fig. 2. Effect of an addition of microbial phytase to a diet based on soy protein isolate and corn starch on the apparent absorption and retention of zinc in growing rats

Table 3. Effect of an addition of microbial phytase to a phytate-rich diet of soy protein isolate and corn starch on plasma zinc-concentration, unsaturated plasma-zinc binding capacity (ZBC) and activity of alkaline phosphatase (ALP) in plasma of growing rats.

Group	Feeding-regimen	PA : Zn (molar)	Phytase (U/kg)		Plasma-Zn (μg/mL)	ZBC (%)	ALP (mU/mL)
I	ad libitum	26 : 1	–	M	0.87[a]	76.0[a]	316[a]
				SD	0.21	6.0	70
II	pair-fed to I	26 : 1	1000	M	1.21[b]	66.2[b]	423[b]
				SD	0.08	2.1	49
III	ad libitum	26 : 1	1000	M	1.15[b]	67.2[b]	400[b]
				SD	0.11	3.2	84

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

Under the conditions investigated, the phytase-supplementation also increased the plasma zinc-concentration and changed the percent unsaturated plasma-zinc binding capacity (Table 3). In accordance with previous experiments (24, 25) a high negative correlation ($r = -0.98$, $p < 0.001$) between both zinc status parameters was observed. Comparable positive effects were obtained for the activity of the zinc metalloenzyme alkaline phosphatase. It has already been reported that molar PA : Zn-ratios > 20 : 1 reduce zinc accumulation in the bones of growing rats (10, 18, 22, 25). In the present study, molar PA : Zn-ratios of 26 : 1 in all three diets were obtained. The addition of 1 000 U of microbial phytase increased the femur zinc-concentration significantly (Table 4) by about 35 % (1 000 U, pair-fed) and 19 % (1 000 U, ad lib.) compared to group I (control). The zinc-concentration in testes similarly increased. In accordance with earlier experiments (22, 25), the liver zinc-concentration remained unchanged. No significant differences could be found between the groups. The retention of other investigated minerals (Ca, P, Mg, Fe, Cu, Mn) also tended to be increased by the phytase-treatment (publication in preparation).

To our knowledge, the influence of an addition of microbial phytase to a soy protein isolate-corn starch-diet on the utilization of zinc in growing rats has not previously been reported. The present study clearly demonstrates that a supplementation of *Aspergillus*-phytase to a phytate-rich diet considerably improves the availability of zinc. Further studies are required to evaluate the effect of a supplementation of microbial phytase on the utilization of other essential elements (Ca, P, Mg, Fe, Cu, Mn) under marginal dietary levels. Interactions between toxic cations (Cd, Pb), phytate (13, 34) and microbial phytase should also be considered.

Table 4. Effect of an addition of microbial phytase to a phytate-rich diet of soy protein isolate and corn starch on zinc-concentration in femur, testes and liver in growing rats

Group	Feeding-regimen	PA : Zn (molar)	Phytase (U/kg)		Femur-Zn (μg/g DM)	Testes-Zn (μg/g DM)	Liver-Zn (μg/g DM)
I	ad libitum	26 : 1	–	M	99.1[a]	139.1[a]	124.6[a]
				SD	17.0	10.2	29.0
II	pair-fed to I	26 : 1	1000	M	133.5[c]	153.5[b]	125.2[a]
				SD	9.8	14.5	16.5
III	ad libitum	26 : 1	1000	M	117.9[b]	151.1[ab]	132.7[a]
				SD	6.5	11.2	17.4

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

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