Journal of Food Technology 3(2) : 188-191, 2005
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Interaction of Dietary Phytate, Calcium and Zinc in Relation to Fat Digestion and Bile Acid Excretion in Rats

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Abstract: The interaction of dietary calcium, phytate and zinc was studied in relation to fat digestion in rats. It was hypothesized that supplemental zinc would nullify the phytate-mediated antagonism of the calcium-induced inhibition of fat digestion. Under in-vitro conditions, the addition of zinc sulphate to a sodium phytate and calcium phosphate containing solution reduced the recovery of bile acid in the supernatant. Rats were fed a diet either in low calcium, high in calcium, high in calcium plus added phytate or high in calcium plus phytate and extra zinc. Addition of phytate to the diet lowered food intake and body-weight gain, but supplemental zinc counteracted these effects. Supplemental zinc in the high-calcium, phytate containing diet increased fecal bile acid excretion, but left fat digestion unchanged. It is concluded that the present data are at variance with the earlier observed negative relationship between fecal bile acid excretion and apparent fat digestibility.

Key words: Phytate, Calcium, zinc, Fat digestion, Bile acid, Rats

Introduction

In a previous study with rats, we found that dietary phytate partially counteracted the calcium-induced inhibition of fat digestion (Yuangklang *et al.*, 2005). It was suggested that phytate competes for calcium with the calcium phosphate sediment in the small intestinal lumen, leading to less calcium phosphate sediment. As a result, less bile acids will be bound to the sediment so that more bile acids become available to participate in the process of fat digestion. Thus, phytate ingestion with a high-calcium diet increases fat digestion and decreases bile acid excretion as was indeed observed (Yuangklang *et al.*, 2005). Phytate is known to form complexes with zinc more efficiently than with calcium (Lönnerdal *et al*, 1989; Graf and Eaton, 1990). It could thus be suggested that high zinc intakes would nullify the phytate-induced antagonism of the calcium-induced inhibition of fat digestion. In other words, high zinc intakes may cause that less calcium will be bound by phytate, leading to more calcium phosphate so that more bile acids are bound and less soluble bile acids are present. As a result, fecal bile acid excretion would be increased and fat digestion be diminished after supplementing a high-calcium, phytate-rich diet with zinc. Thus, we hypothesized that excessive zinc feeding would oppose the effect of phytate in counteracting the calcium-induced lowering of fat digestion. The hypothesis was tested in the present in-vitro experiment and in a feeding trial with rats.

Materials and Methods

In-Vitro Experiment: To check whether zinc would counteract the effect of phytate on bile acid solubility, in-vitro experiments were carried out. Zinc sulphate was added at levels of 0, 12, 24, 48 or 96 ppm to a 0.075 % sodium phytate solution of 22 mM of $CaHPO_4$; the total volume was 25 ml. Then the solution was adjusted to pH 2.0 by using 6 M HCl, and 250 il of pepsin solution (4 mg/ml) was added. The mixture was incubated at 37 °C for 40 min and then cooled down and adjusted to pH 7.0 with NaOH. Under constant stirring conditions, the mixture was pipeted into an Eppendorf cup (4 cups per treatment). Subsequently, 10 il of mixed micelle solution (40 mM cholesterol, 120 mM phospholipids and 360 mM glycodeoxycholate) and 10 il of pancreatin solution (24 mg/ml) was added to the cups. The cups were then incubated in a water bath at 37 ?C for 2 hours and vortexed several times during incubation. After 2 hours, the solution was cooled down and centrifuged at 14000 x g for 10 min in an Eppendorf-cup centrifuge. Then, the supernatant was diluted with demineralized water. The diluted supernatant was then measured for bile acids on a Cobas-Mira Centrifugal analyzer with the bile acid kit purchased from Sigma Diagnostics.

Feeding Trial wih Rats: The protocol of the experiment was approved by the animal experimentation committee of the Utrecht Faculty of Veterinary Medicine. Forty-eight outbred male Wistar rats (U:WU, Utrecht University) were used. On arrival, the rats, which were aged 3 weeks, were housed in groups of two in polycarbonate cages with sawdust as bedding. The rats were fed ad libitum on a commercial, pelleted diet and tap water for 3 days. At the end of the pre-experimental period (day 0), the rats were divided into four groups of 12 rats each so that body

weight distributions of the groups were similar. The groups were randomly allocated to the one of the four experimental diets (Table 1).
The rats were housed individually in metabolic cages as described (Yuangklang *et al.*, 2005). The experiment lasted 25 days. The rats were weighed at the beginning and end of the experiment. Feed intakes were measured. From days 14 to 24, each day feces were collected quantitatively and subsequently pooled per rat and stored at -20 °C.

Chemical Analyses: Feces samples were dried at 60 °C for 48 hours. Diet and feces samples were analyzed for total lipids, dry matter, crude protein and ash by the Weende method. Calcium, phosphorus and zinc in diet samples were analysed as described (Yuangklang *et al.*, 2004). Feces samples were extracted and analyzed by an enzymatic method for total bile acids (Yuangklang *et al.*, 2004).

Statistical Analyses: The data were statistically analyzed with Tukey's multiple comparison test using a computer program (SPSS for Windows 9.0, SPSS Inc., Chicago, IL, 1998). The level of significance was pre-set at $p<0.05$.

Results

In-Vitro Solubility of Bile Acids: With added sodium phytate, but without added zinc sulphate, the recovery of glycodeoxycholate in the supernatant fraction was 100 % (Fig. 1). Addition of zinc sulphate to the phytate and calcium-phosphate containing solution reduced the recovery of the bile acid. This in-vitro experiment indicates that zinc addition counteracts the phytate-induced increase in bile acid solubility, which would be anticipated to depress fat digestibility and raise bile acid excretion in the feeding trial with rats. More explicitly, it would be expected that excessive zinc consumption antagonizes the relieving effect of phytate on the calcium-induced inhibition of fat digestion.

Body Weight and Feed Intake: The addition of phytate to the high-calcium diet lowered feed intake when compared with the other diets (Table 2). Body-weight gain was lowest in the phytate group without supplemental zinc, this effect being counteracted by zinc supplementation. Possibly, phytate feeding without extra zinc had caused zinc deficiency which is known to depress food intake and to reduce weight gain. High versus low-calcium intake raised body-weight gain and also feed intake, which is in agreement with earlier work (Yuangklang *et al.*, 2005).

Apparent Digestibility of Nutrients: Table 3 shows that rats fed the low-calcium diet had highest nutrient digestibilities. Rats fed the high-calcium diet had significantly lower apparent fat digestibility than rats fed the low-calcium diet. Fat digestion was raised when the high calcium intake was combined with phytate. Zinc supplementation of the high-calcium, phytate-rich diet had no effect on fat digestibility. The addition of zinc sulphate to the diet increased protein digestibility.
Bile Acid Excretion: Rats fed the high-calcium diet showed higher group-mean fecal bile acid excretion than rats fed the low-calcium diet (Table 3). In rats fed the high-calcium diet with added phytate, bile acid excretion in feces was reduced when compared to rats fed the high-calcium diet without phytate (Table 3). The addition of zinc to the high-calcium, phytate-rich diet significantly elevated fecal bile acid excretion.

Table 1: Ingredient and analysed composition of the experimental diets

	Low calcium	High calcium	High calcium + 0.8 % phytate	High calcium +0.8 % phytate+ zinc
Ingredients, g/kg				
Constant components[1]	477	477	477	477
Corn starch	516.8	498.2	490.2	489.87
Calcium carbonate	6.2	24.8	24.8	24.8
Zinc sulphate	0	0	0	0.33
Sodium phytate	0	0	8	8
Analyzed composition				
Dry matter, g/100 g	93.9	93.6	94.2	93.9
Crude protein, g/100 g	18.1	18.0	17.9	18.0
Total lipids, g/100 g	21.0	20.9	21.0	21.2
Calcium, g/kg	2.77	9.36	9.37	9.38
Phosphorus, g/kg	4.76	4.98	6.21	6.09
Zinc, mg/kg	21	20	18	145

[1]The composition of the constant components was as follows (g): casein, 200; animal fat, 180; soybean oil, 20; cellulose, 30; $NaH_2PO_4.2H_2O$, 15; $MgCO_3$, 2; KCl, 8; mineral premix, 10; vitamin premix, 12. The composition of the mineral and vitamin premixes has been described (Terpstra *et al.*, 1998).

Table 2: Body weight and food intake of rats fed the experimental diets

	Low calcium	High calcium	High calcium + 0.8 % phytate	High calcium +0.8 % phytate+zinc
Body weight, g				
Initial	67.4 ± 9.1	66.8 ± 9.0	68.4 ± 9.8	67.9 ± 9.5
Final	211 ± 19.6^{A}	229 ± 19.1^{A}	159 ± 14.7^{B}	227 ± 19.2^{A}
Body-weight gain, g	5.99 ± 0.64^{B}	6.77 ± 0.56^{A}	3.76 ± 0.74^{C}	6.65 ± 0.56^{AB}
Food intake, g/d	14.5 ± 1.2^{B}	16.3 ± 1.4^{A}	9.36 ± 1.2^{C}	16.1 ± 1.5^{A}

Means ± SD for 12 rats per dietary group.
^{ABC}Means in the same row not sharing a common superscript are significantly different ($P<0.05$).

Table 3: Nutrient digestibility and bile acid excretion in rats fed the experimental diets

	Low calcium	High calcium	High calcium + 0.8 % phytate	High calcium +0.8 % phytate+zinc
Digestibility, % of intake				
Dry matter	96.5 ± 0.21^{A}	93.3 ± 0.31^{B}	93.3 ± 0.69^{B}	93.3 ± 0.63^{B}
Crude protein	97.0 ± 0.29^{A}	95.1 ± 0.30^{B}	94.5 ± 0.55^{C}	95.2 ± 0.56^{B}
Ash	95.0 ± 0.54^{A}	74.5 ± 2.0^{B}	70.4 ± 3.0^{C}	72.6 ± 2.6^{BC}
Fat	97.4 ± 0.21^{A}	90.3 ± 0.76^{C}	92.5 ± 1.0^{B}	91.7 ± 0.77^{B}
Fecal bile acid excretion, µmol/day	6.4 ± 0.86^{AB}	7.1 ± 0.95^{A}	5.4 ± 1.2^{B}	7.2 ± 1.3^{A}

Means ± SD for 12 rats per dietary group.
^{ABC}Means in the same row not sharing a common superscript are significantly different ($P<0.05$).

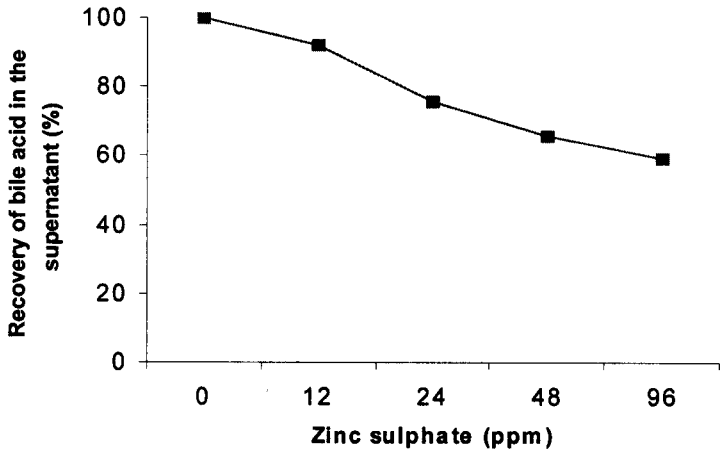

Fig. 1: The solubility of glycodeoxycholate in in-vitro incubations at different levels of zinc in a mixture containing calcium phosphate and sodium phytate. Recovery in the supernatant fraction is expressed as a percentage of the total amount of bile acids in the incubation.

Discussion

In a previous experiment (Yuangklang *et al.*, 2005) it was shown that phytate added to a high-calcium diet enhanced fat digestion and depressed the excretion of bile acids in feces. In this experiment we tested whether zinc feeding would counteract the effect of phytate. The present data confirm that phytate supplementation of the high-calcium diet significantly enhanced fat digestion and depressed the excretion of bile acids in feces. However, the addition of zinc to the high-calcium, phytate containing diet did not influence fat digestion, whereas it significantly raised the excretion of bile acids in feces. This outcome cannot be readily explained. The excretion of bile acids and fat digestibility are negatively correlated (Xu *et al.*, 2001). The zinc effect on bile acid excretion supports our hypothesis, but the associated lack of effect on fat digestibility does not. The in-vitro experiment showed that the phytate-mediated increase in bile acid solubility was antagonized by the addition of zinc. A lower solubility of bile acids may cause a lower re-absorption, leading to a higher fecal excretion of bile acids. Thus, it was indeed expected that supplemental zinc in a high-calcium, phytate containing diet would increase fecal bile acid excretion.

Acknowledgement
The authors wish to thank Marjan Javadi, Robert Hovenier and Jan van der Kuilen for analytical assistance and Anja van der Sar and Joyce Visser for biotechnical help.

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