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Sodium phytate counteracts the inhibitory effect of calcium on fat digestion in rats

[1]C. Yuangklang, [1]Th. Wensing,[2] A. G. Lemmens,[2] S. Jittakhot, and[2] A.C. Beynen
[1]Department of Nutrition and [2]Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract: The objective of the present experiment was to investigate whether phytate feeding would counteract the inhibitory effect of calcium on fat digestion in rats. Rats were fed semipurified diets either low in calcium or high in calcium with various levels of added sodium phytate. Body weight and food intake were not influenced when phytate was added to the high-calcium diet. Phytate significantly raised fat digestibility and diminished group mean fecal bile acid excretion when compared to the high-calcium, phytate-free diet. The apparent digestibilities of dry matter, crude protein and ash were not affected by phytate. It is suggested that phytate feeding counteracts the inhibitory effect of calcium on fat digestion through complexing calcium in the small intestinal digesta, leading to less calcium phosphate sediment so that less bile acids are bound to the sediment and more bile acids were available for the process of fat digestion.

Keywords: Phytate, calcium, fat digestion, bile acid excretion, rats

Introduction

In in-vitro experiments, bile acids are bound by a precipitate of calcium phosphate, but the addition of phytate increased the solubility of bile acids (Yuangklang *et al.*, 2004a). Studies in rats, rabbits and calves (Beynen *et al.*, 2002; Van der Meer *et al.*, 1985; Xu *et al.*, 1998) showed that high calcium intakes depressed fat digestion and elevated bile acid excretion in feces. It was suggested that high calcium intakes increase the amount of insoluble calcium phosphate in intestinal digesta which binds bile acids and thereby withdraws them from the process of fat digestion. Phytate binds calcium (Heaney *et al.*, 1991). Thus, it could be hypothesized that the feeding of phytate would counteract the calcium-induced inhibition of fat digestion in rats. To test this hypothesis, rats were fed high-calcium diets supplemented with different phytate levels.

Materials and Methods

Feeding trials with rats: The experimental protocol was approved by the animal experiments committee of the Utrecht Faculty of Veterinary Medicine. Sixty outbred maleWistar rats (U:WU, University Utrecht, The Netherlands) 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 at ad libitum on a commercial, pelleted diet and tap water for 5 days. At the end of the pre-experimental period (day 0), the rats were divided into five 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 five experimental diets (Table 1).

The rats were housed individually in metabolic cages. The cages were placed in a room with controlled temperature (20-22 °C), relative humidity (45-55%) and lighting (0700-1900 h). The experimental diets and demineralised water were offered ad libitum. The experiment lasted 25 days. The rats were weighed at the start and end of the experiment. Feed intakes were measured. From days 14 to 24, daily 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, ash and crude protein according to the Weende method. Calcium and phosphorus in diet samples were analysed as described (Xu *et al.*, 1998). Feces samples were extracted and analysed by an enzymatic method for total bile acids (Yuangklang *et al.*, 2004b).

Statistical Analyses: The data were statistically analyzed with Tuckey' 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

There was no significant difference in final weight and body-weight-gain for the experimental diets. Rats in the low-calcium group had a significantly lower food intake than those in the other groups.

Digestibilities of dry matter, crude protein, total fat and ash in rats fed the low-calcium diet were significantly higher than in rats fed the other diets (Table 3). Apparent digestibilities of dry matter, crude protein and ash were not influenced by supplemental phytate, but fat digestibility was significantly enhanced. Inclusion of phytate at 0.8

and 1.2% in the high-calcium diets produced significantly increased fat digestibilities, when compared with high

Table 1. Ingredient and analysed composition of the experimental diets

	Experimental diets				
	1*	2	3	4	5
Ingredients, g/kg					
Constant components[1]	477	477	477	477	477
Corn starch	516.8	498.2	494.2	490.2	486.2
$CaCO_3$	6.2	24.8	24.8	24.8	24.8
Na-phytate	0	0	4	8	12
Analyzed composition, g/100 g					
Dry matter	91.1	91.8	91.6	91.5	91.6
Crude protein	16.3	17.0	16.9	16.5	17.1
Crude fat	20.0	20.2	20.0	20.0	20.1
Ash	2.92	4.47	4.69	4.96	5.10
Calcium	2.98	8.68	8.77	8.70	8.85
Phosphorus	4.31	4.17	4.77	5.26	5.98

[1]The constant components consisted of the following (g):casein, 200; animal fat, 180; soya 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).
*1 = low calcium; 2 = high calcium; 3 = high calcium + 0.4 % phytate; 4 = high calcium + 0.8 % phytate; 5 = high calcium + 1.2 % phytate

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

	Experimental diet				
	1	2	3	4	5
Body weight, g					
Initial	65 ± 7.6	67 ± 7.5	67 ± 7.9	65 ± 8.3	66 ± 8.8
Final	209 ± 10	222 ± 9.6	218 ± 12	213 ± 18	219 ± 16
Body weight gain, g/d	6.0 ± 0.4	6.5 ± 0.4	6.3 ± 0.5	6.2 ± 0.6	6.4 ± 0.6
Food intake, g (d 23-25)	30.5 ± 1.1^{A}	32.4 ± 0.4^{B}	33.0 ± 0.8^{B}	31.8 ± 0.9^{B}	32.7 ± 0.4^{B}

Means ± SD for 12 rats per dietary group.
*1 = low calcium; 2 = high calcium; 3 = high calcium + 0.4 % phytate; 4 = high calcium + 0.8 % phytate; 5 = high calcium + 1.2 % phytate.
[AB]Mean values in the same row with different superscript letter are significantly different: $P<0.05$

Table 3. Apparent digestibilities of macronutrients and fecal bile acid excretion in rats fed the experimental diets

	Experimental diet				
	*1	2	3	4	5
Digestibility (% of intake)					
Dry matter	94.0 ± 0.4^{A}	89.8 ± 0.4^{BC}	89.7 ± 0.5^{C}	89.4 ± 0.5^{C}	89.8 ± 0.3^{BC}
Ash	86.5 ± 1.9^{A}	56.0 ± 2.1^{B}	56.8 ± 2.1^{B}	56.8 ± 1.3^{B}	57.3 ± 1.5^{B}
Crude protein	94.5 ± 0.4^{A}	91.7 ± 0.6^{B}	91.9 ± 0.7^{B}	92.2 ± 0.7^{B}	92.5 ± 0.5^{B}
Total lipids	92.4 ± 0.7^{A}	76.8 ± 1.3^{B}	77.6 ± 1.2^{BD}	78.6 ± 1.2^{CD}	79.6 ± 1.0^{C}
Fecal bile acid excretion (μmol/day)					
	6.9 ± 1.7	8.6 ± 2.1	8.1 ± 2.5	7.6 ± 2.5	6.8 ± 1.7

Means ± SD for 12 rats per dietary group
*1 = low calcium; 2 = high calcium; 3 = high calcium + 0.4 % phytate; 4 = high calcium + 0.8 % phytate; 5 = high calcium + 1.2 % phytate
[ABCD]Mean values in the same row with different superscript letter are significantly different: $P<0.05$

calcium intake alone. Rats fed cholate showed a significantly increased fat digestion when compared to their counterparts fed one of the high-calcium diets.
Increasing dietary phytate levels were related with bile acid excretion in feces, but the phytate effect was not statistically significant (Table 3). There was no significant difference between rats fed either the low or high-calcium diet, but group-mean bile acid excretion was increased by 25% in rats fed the high-calcium diet.

Discussion

The objective of the present experiment was to investigate whether phytate feeding would counteract the calcium-induced inhibition of fat digestion in rats. The data clearly show that phytate intake with a high-calcium diet elevated fat digestion and depressed fecal bile acid excretion in a dose-dependent fashion. The phytate-induced increase in apparent fat digestibility became significant at inclusion levels of 0.8 and 1.2 %. The phytate-induced decrease in bile acid excretion did not reach statistical significance. Nevertheless, it would appear that our hypothesis described above is supported by the present data. The observations corroborate the earlier reported (Xu *et al.*, 2001) negative association between fat digestibility and fecal bile acid excretion. It appears that the solubility of bile acids in the intestinal digesta is a major determinant of the efficiency of fat digestion and the fecal excretion of bile acids.

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