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Effects of dietary phytic acid on digestibility of main nutrients and mineral absorption in mink (*Mustela vison*)*

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ABSTRACT

The objective of the study was to evaluate the effects of dietary phytic acid (IP6) on the apparent total tract digestibility (ATTD) of main nutrients and apparent absorption of minerals in mink *(Mustela vison)*. A basal moist diet consisting of fish meal, fish oil and wheat starch was added at different concentrations of soluble sodium phytate, thus obtaining six diets with graded levels of IP6 (0-19.9 g IP6 kg dry matter^{-1}). Each diet was fed to four adult male mink kept in individual cages. There were no significant effects of IP6 on the ATTD of crude protein, fat, starch or energy, whereas the apparent absorption of copper and magnesium was significantly reduced with increasing concentration of IP6. Thus, as observed in other monogastric animals, we conclude that dietary IP6 could impair mineral status in mink, but may not affect digestibility of energy-yielding nutrients.

KEY WORDS: mink, phytic acid, nutrients, minerals

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INTRODUCTION

Phytic acid (*myo*-inositolhexaphosphate, IP6) is the storage form of phosphorus (P) in plants and feed ingredients of plant origin. The molecule consists of an inositol ring with six phosphate groups covalently bound to the ring structure. The phosphate groups are highly reactive and have a strong affinity to divalent and trivalent minerals (Persson et al., 1998). In its natural form, IP6 is often found as a phytate, which is a salt of IP6. In contrast to ruminants, monogastrics lack, or have very low endogenous activity of the enzyme phytase, which is needed for hydrolysis of IP6 to yield lower inositol phosphates and free phosphate. Besides from lowering the availability of P and other minerals in monogastrics, IP6 may reduce the availability of main nutrients such as protein, starch and lipids, apparently *via* its ability to reduce the activity of digestive enzymes (Singh and Krikorian, 1982). Thus, high levels of phytate have frequently been assumed to explain a low digestibility and utilization of main nutrients. However, there are other reports where IP6 or the addition of phytase to IP6-containing diets have had no effect on the digestibility of main nutrients and energy in pigs (Liao et al., 2005), poultry (Peter and Baker, 2001) or fish (Denstadli et al., 2006). However, besides from a study with salmon (Denstadli et al., 2006), there are no dose response studies available on dietary IP6 supplementaton.

Although the mink *(Mustela vison)* is a carnivorous animal species, the NRC recommends that carbohydrates provide 15-25% of total metabolizable energy. Thus, cereals and supplementary plant protein sources may contribute up to 50% of dietary dry matter. Inclusion of plant-derived feed ingredients with inherent IP6 in diets for mink may therefore be of importance for mineral availability and possibly for digestibility of main nutrients. Moreover, the digestibility of main nutrients in mink may correlate well with that of salmon, pig and chicken (Skrede et al., 1998), and would also serve as an indicator of digestibility in dogs (Ahlstrøm and Skrede, 1998).

The aim of the present experiment was to study how graded levels of phytic acid (0-19.9 g IP6 kg DM^{-1}) added to a basal diet containing fish meal, fish oil and wheat starch affect the digestibility of main nutrients and absorption of minerals in mink.

MATERIAL AND METHODS

Animals and diets

The experiment was carried out at the Center of Animal Research located at the Norwegian University of Life Sciences, Ås (Norway). All mink were cared for according to laws and regulations controlling experiments with live animals in Norway (Animal Protection Ordinance concerning experiment with animals of 15th January, 1996). An extruded diet containing fish meal, wheat starch and vitamin/mineral premix was produced at FôrTek - Center for Feed Technology (Norwegian University of Life Sciences, Ås, Norway). The dry extruded diets were added fish oil and water and soaked overnight, obtaining a porridge-like consistency and approximately 40% dry matter (DM). The formulation of the basal diet is shown in Table 1. From this, six moist diets were made by adding six different levels of solubilized sodium phytate (P8810, Sigma-Aldrich, St.Louis, MO, USA) to obtain planned concentrations of 0, 1.5, 3, 6, 12 and 24 g IP6 kg DM^{-1}. Sodium phytate was thus added at levels of 0, 2.1, 4.2, 8.3, 16.7 and 33.4 g kg DM^{-1}. The analysed chemical composition is shown in Table 2. Individual daily rations of 180 g were prepared and stored at -22°C until experimental start.

Table 1. Ingredient composition of basal diet, g kg^{-1}

Fish meal	688.5
Wheat starch	145.6
Fish oil	165.1
Vit/min premix [1]	0.68

1 per kg diet, IU: vit. D_3, 3400; mg: α-tocopherol 38.5, vit. K 2.27, ascorbate polyphosphate 22.7, vit. B_1 2.27, vit. B_2 4.53, vit. B_6 2.26, vit. B_{12} 0.002, pantothenic acid 9.1, niacin 3.4, biotin 0.04, folic acid 1.81, $ZnSO_4$ 34, $MnSO_4$ 11.3, $CuSO_4$ 2.27

Table 2. Analysed chemical composition of experimental diets

	1	2	3	4	5	6
Dry matter, g kg^{-1}	373	373	373	372	373	387
In dry matter, kg^{-1}						
protein (N × 6.25), g	541	533	539	536	535	517
lipid, g	241	242	234	237	233	235
starch, g	109	108	108	106	112	107
ash, g	104	112	107	108	113	121
energy, MJ	23.5	23.4	23.4	23.3	23.2	22.8
phosphorus, g	16.2	16.6	16.9	18.0	19.6	22.5
calcium, g	23.9	24.0	23.5	24.0	23.7	23.1
magnesium, g	1.63	1.63	1.63	1.64	1.64	1.60
zinc, mg	94	97	93	91	90	89
iron, mg	122	138	138	165	165	122
copper, mg	7	9	7	9	7	7
IP6[1], g	0	1.1	2.7	4.7	10.1	19.9

[1] *myo*-Inositol hexaphosphate (IP6)

Four randomly picked healthy standard brown male mink (approximately 18 months old, weight range: 2.4-3.3 kg) were assigned to each of the six diets. The animals were kept in individual cages in a soundproof room with a constant temperature of 13°C. The lights were automatically adjusted to the outside diurnal conditions *via* a photocell. The cages were equipped for controlled feeding and separate collection of faeces and urine according to Jørgensen and Hansen (1973). The digestibility experiment lasted for seven days, of which three days of adaptation were followed by four days of faecal collection. Faeces from each individual mink were stored at -22°C after each daily collection.

Chemical analyses

Freeze-dried samples of diets and faeces were analysed for dry matter (drying at 104°C for 16 h), ash (determined gravimetrically after combustion at 550°C for 16 h), crude protein (Kjeldahl-N × 6.25) and crude fat (HCl hydrolysis with petroleum ether extraction) as described by AOAC (2000). Starch was analysed as described by McCleary et al. (1994). For elemental analysis, ground and pre-ashed samples were added a mixture of HNO_3 and HCl (2:1, v/v), and boiled until the colour changed from yellow to colourless. The samples were diluted with distilled water and further analysed using an ICP-AES. The method described by Carlsson et al. (2001) was used for the determination of IP6.

Calculations and statistical analyses

The apparent total tract digestibility (ATTD) of main nutrients and absorption of minerals were calculated from analytical figures and the quantitative measurement of feed intake and faecal output. The data were analysed by regression analysis according to the best relative fit using linear ($y = \beta_0 + \beta_1 x + \varepsilon$) or quadratic ($y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon$) models, where β_0 is the intercept, and β_1 and β_2 represent the linear and quadratic parameters for the fixed effect of IP6, respectively, and ε as the random effect. SAS software version V8 (SAS Institute, Cary, NC, USA) was used to analyse the experimental data.

RESULTS

The chemical analyses of the diets revealed an increase in content of phosphorus as a result of the increased addition of sodium phytate. The experiment was carried out without problems and feed intake was normal for all animals except one animal given diet 6, which was omitted from the experiment due to poor feed consumption. A subjective evaluation of faecal consistency indicated no differences among animals fed the different diets, and no animals exhibited loose or diarrhoea-like faeces. Dietary IP6 had no significant effect on feed intake.

The ATTD of main nutrients and energy is presented in Table 3. There was no difference among diets in the apparent digestibility of crude protein, lipid, starch or energy, and there was minor variation within diet groups. The apparent absorption of minerals (Table 3, Figure 1) was subject to large individual variation, and several negative apparent absorption values appeared. There was a significant quadratic effect of IP6 on the apparent absorption of Cu and Fe, and significant linear effects on apparent absorption of Mg and Ca. The highest IP6 inclusion levels tended to lower the apparent absorption of Mg and Ca. The apparent absorption coefficients for Zn were highly negative for all animals (average value: -138%).

Table 3. Effect of increased levels of IP6 on apparent total tract digestibility (%) of main nutrients and apparent total tract absorption (%) of minerals in mink (n=4)

Nutrients	Diets						SEM	Regression	
	1	2	3	4	5	6[1]			
	0 g IP6 kg^{-1}	1.1 g IP6 kg^{-1}	2.7 g IP6 kg^{-1}	4.7 g IP6 kg^{-1}	10.1 g IP6 kg^{-1}	19.9 g IP6 kg^{-1}		equation	R^2/ significance
Protein	87.8	87.2	87.5	87.5	87.9	87.3	0.45		NS
Fat	97.4	97.7	97.3	97.6	97.6	97.8	0.15		NS
Starch	99.6	99.7	99.7	99.6	99.6	99.7	0.05		NS
Ash	28.7	32.3	28.9	30.4	34.9	34.2	1.60		NS
Energy	90.9	90.6	90.5	90.5	90.8	90.5	0.34		NS
Phosphorus	16.1	22.4	9.9	26.4	20.6	23.0	3.2		NS
Calcium	-6.3	-1.0	-14.2	2.6	-7.2	-22.1	4.4	y=-2.9-0.8x	0.17[2]
Magnesium	6.1	9.8	-10.8	12.0	-2.5	-11.0	4.3	y =5.8-0.8x	0.17[2]

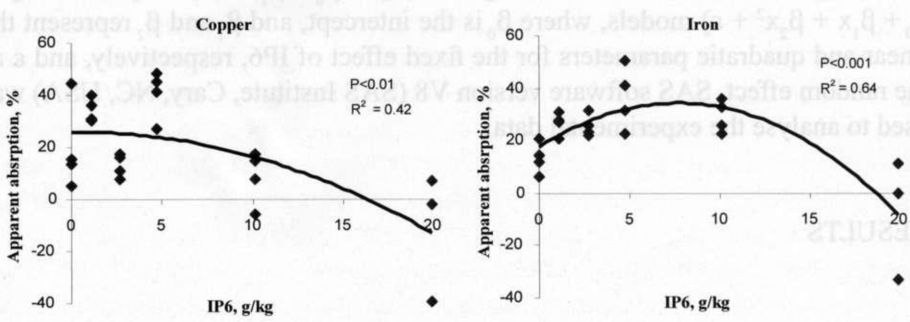

Figure 1. Apparent absorption (%) of copper (Cu) and iron (Fe) in mink fed graded levels of phytic acid (IP6)

DISCUSSION

Apparent digestibility of main nutrients. As outlined in the introduction, the negative effects of IP6 on mineral absorption are well established in several monogastric species, whereas the effect on digestibility of main nutrients seems to be more variable. To our knowledge there are no published data on effects of IP6 or other inositol phosphates on the digestibility or absorption of nutrients in mink. In the present study digestibility of protein, lipid, starch and energy remained unaffected by the concentration of IP6 in the diet. These results are in line with other investigations with pigs (Liao et al., 2005), poultry (Peter and Baker, 2001) and fish (Denstadli et al., 2006), but contradict with other studies with fish (Spinelli et al., 1983) and poultry (Ravindran et al., 2000). Most studies carried out so far have aimed at evaluating the effects of IP6 on protein and energy utilization. In studies where IP6 affected utilization of main nutrients, the formation of IP6-enzyme or other IP6-protein complexes have been hypothesized as the most plausible explanations. *In vitro* assay systems could clarify this, but while some *in vitro* studies have shown that IP6 reduced the trypsin activity (Singh and Krikorian, 1982), others have failed to find any effect on trypsin (Deshpande and Damodaran, 1989). The results from *in vitro* studies are sensitive to variation in experimental conditions such as pH, incubation time, substrate and presence of cations such as Ca. Moreover, *in vivo* trials have shown that the trypsin activity does not necessarily conform with protein digestibility (Sajjadi and Carter, 2004). Likewise, Yuangklang et al. (2005) concluded from a study where sodium phytate had an effect on bile acids *in vitro*, that there was no relationship with *in vivo* lipid digestibility in rats. The use of different protein sources in different studies may also explain contradictory effects of IP6 on utilization of main nutrients (Kies et al., 2006) The solubility profile of protein from different sources such as soya, canola, sunflower and maize vary with pH levels, and the presence of IP6 has a significant effect on the solubility of protein. Thus, in a study carried out by Kies et al. (2006) the solubility of soya protein was reduced from 91 to 2% at pH 2, and from 60 to 23% at pH 3, when IP6 was added to the solution. In contrast, the solubility of canola protein was unaffected by IP6 at pH 3. This indicates that gastric pH and type of protein must be taken into consideration when effects of IP6 are evaluated. In a study by Szymeczko and Skrede (1990) a gastric pH of 3.7 was found in mink 90 min after feeding a fish meal based diet, declining to a pH of 3.3 after 180 min. Based on this we suggest that proteins of animal origin are less susceptible to reduced protein solubility than globular soya proteins in the presence of IP6.

Apparent absorption of minerals. Assessing the absorption of minerals is a complex matter, considering the numerous interacting factors that can influence the results. The endogenous contribution, such as bilary and gastrointestinal secretions and sloughed mucosal cells (Sandström et al., 1993), should also be taken into consideration. To date there are few reports on mineral absorption and homeostasis in terrestrial carnivorous species such as the mink. In the present study, the apparent absorption of minerals showed large individual variation, which is in line with other studies on species such as polar foxes (Szymeczko et al., 2005) and dogs (Hill et al., 2001). High individual variation was also found when radiolabelled Fe was used to assess uptake of iron in mink (Skrede, 1970). In our study, repeated analyses revealed that great individual variation was the main reason for the high SEM for apparent absorption of minerals.

A gradual decrease in the apparent absorption of Cu with increasing levels of IP6 (Figure 1), agrees with results obtained by Davies and Nightingale (1975) where a purified diet added sodium phytate (10 g kg^{-1}), reduced whole body retention of Cu in male rats. IP6 has a strong affinity to Cu, and as demonstrated by Persson et al. (1998), Cu is practically insoluble at pH 7 in the presence of IP6. A reduction in the apparent absorption of Mg due to IP6 has been shown in studies with rats (Pallauf et al., 1998) and salmon (Denstadli et al., 2006). The apparent absorption of Mg, Fe and Ca was significantly affected by the diet, but there was no clear dose response effect related to the inclusion levels of IP6. Even though the chelating capacity of the IP6 molecule is indisputable, the molecule cannot bind to more cations than there are spare seats left in the phosphate groups, and the binding capacity depends on plant origin of IP6. Except for the highest inclusion level (19.9 g IP6 kg^{-1}), where the apparent absorption of Fe dropped markedly, levels up to 10 g IP6 kg^{-1} had no negative effect. Thus, our results would tend to support the findings by Graf and Eaton (1984) working with mice, that moderate concentrations of IP6 had no detrimental effects on duodenal absorption of Fe and Ca. Previous studies with Atlantic salmon revealed declining Zn absorption with increasing IP6 levels (Denstadli et al., 2006). In the present study the apparent Zn absorption was highly negative (not presented), probably due to contamination from Zn galvanized toy equipment in the cages.

CONCLUSIONS

To conclude, purified IP6 had no effect on the digestibility of main nutrients, but reduced the apparent absorption of Cu and Mg in mink. In further studies, we suggest that the effects of IP6 on mineral availability should be evaluated in long term *in vivo* studies involving analyses of plasma mineral status and *post mortem* bone tissue histology and mineral analysis.

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