Effects of supplemental phytase on nutrient excretion and retention in broilers fed different cereal based diets

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ABSTRACT: A 21-day experiment with day-old broilers was conducted in order to assess the effect of phytase supplementation to different cereals-soybean meal based diets on nitrogen, calcium, total and phytate phosphorus excretion and retention. Diets were formulated to contain 4 different cereals (maize, wheat, triticale and barley), 2 levels of dietary calcium (0.6 and 1.0%) and 3 levels of supplemental phytase (0, 500 and 1 000 PU/kg). Nutrient intake, excretion and retention were determined in the first three days of the 2nd and 3rd week of trial. The increasing phytase level resulted in reducing phytate phosphorus excretion and increasing its retention. Significant differences were obtained only in the 2nd week (excretion $P < 0.024$ and retention $P < 0.044$). Phytase supplementation significantly influenced total phosphorus excretion in the 3rd week ($P < 0.048$) and retention in the 2nd week ($P < 0.015$). The effects of 500 and 1 000 PU/kg were not statistically different from each other. No significant influence of phytase supplementation on nitrogen and calcium excretion and retention was observed.

Keywords: maize; wheat; triticale; barley; phytase; nitrogen; calcium; total phosphorus; phytate phosphorus; excretion; retention

Reduction of phosphorus excretion into the environment has become one of the most important tasks in modern poultry production. As it is a mineral essential for poultry growth, it is of special interest that diets fulfil poultry needs for phosphorus. Poultry diets are usually based on cereals in which 50–70% of total phosphorus is bound in the form of phytate, which is unavailable to the poultry due to the lack of the enzyme phytase. Additionally, nutritional problems arise because phytate makes complexes with minerals (calcium, magnesium, iron and zinc) and proteins (Enzymes in Animal Nutrition, 1998). Ca-phytate complexes bind starch (Thompson and Yoon, 1984; Thompson, 1988) and inhibit α-amylase activity (Deshpande and Cheryan, 1984), reducing solubility and digestibility of starch in this way. Phytates also form insoluble complexes with fatty acids and inhibit pepsin activity.

Nowadays two approaches to solving this problem dominate: addition of phytase, enzyme that hydrolyses phytate acid, or using genetically modified cereals with lower phytate phosphorus content (Li *et al.*, 2000; Waldroup *et al.*, 2000).

Supplementation of enzymes to animal diets has been approved because they are natural fermentation products and therefore they do not pose a threat to animal and human health (Guenter, 1997). Supplemental enzymes are digested in the same way as other proteins and their residues are not present in the excreta (Enzymes in Animal Nutrition, 1998).

Numerous studies have shown beneficial effects of phytase supplementation on total and phytate phosphorus, calcium and nitrogen retention and excretion in broilers fed maize-soybean meal based diets (Sebastian *et al.*, 1996a, 1997; Simons *et al.*, 1990; Broz *et al.*, 1994; Yi *et al.*, 1996) but investigations with diets based on other cereals are scarce. Ravindran *et al.* (1999) showed that supplementation of phytase (1 200 PU/kg) improved the digestibility of protein and amino acids in maize, sorghum and wheat based diets. The magnitude

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of response varied between cereals. Supplemental phytase improved phosphorus retention in broilers fed wheat (Leske and Coon, 1999; Zyla *et al.*, 2000), wheat midds, barley, defatted rice bran and canola based diets (Leske and Coon, 1999).

The aim of this investigation was to determine and compare the effects of phytase supplementation to diets based on maize, wheat, barley and triticale on the broiler performance, nutrient retention and excretion, as well as bone mineralization. In the preceding paper we showed that the magnitude of beneficial effect of phytase supplementation on broiler performance varied between different cereals and calcium levels (Pintar *et al.*, 2004). In this paper the effects on nitrogen, calcium, total and phytate phosphorus excretion and retention are presented.

MATERIAL AND METHODS

Materials and manipulation. Two hundred forty-five day-old commercial male broiler chicks (Ross) were randomly assigned to 24 pens. Chickens were weighed on Mettler electrical balance (± 0.1 g). Average body weights in different groups were not statistically different.

The chicks were maintained in wire-floored pens with separated excreta trays, placed in an environmentally controlled room. The birds received constant electrical illumination.

Diets. The study was conducted as a 4 × 3 × 2 factorial arrangement of treatments to evaluate the effects of four cereals (maize, wheat, barley and triticale), three levels of supplemental phytase (0, 500 and 1 000 PU/kg) and two levels of calcium (0.6 and 1.0%) on broiler response. Feed and water were made available for *ad libitum* consumption.

Diet ingredients were obtained in bulk. The Novo CT (Novo Nordisk) phytase was used. Both ingredients and diets were analysed for moisture, ash, protein, fat, fibre, calcium and total phosphorus by usual methods (ISO 6490-1, 1985; ISO 5983, 1997; ISO 6491, 1998; ISO 6496, 1999; ISO 5498, 1999; ISO 6492, 1999; ISO 5984, 2002). Phytate phosphorus content was determined according to Sooncharernying and Edwards (1993). In brief, phytate was selectively precipitated with $FeCl_3 \times 6\,H_2O$ from an extract obtained after 24 hours of sample extraction with the solution of HCl and Na_2SO_4. The precipitate was degraded in a mixture of concentrated H_2SO_4 and HNO_3 (2 : 3). The content of phytate phosphorus in the obtained solution was determined by the usual spectrophotometric method for phosphorus determination (ISO 6491, 1998). Compositions of diets are given in Table 1.

Retention and excretion. During the first three days of the 2nd and 3rd week of trial all excreta were collected from each pen and feed consumption was determined. Excreta were stored in plastic bags at –20°C. Before chemical analysis samples were defrosted, homogenized and dried at 60°C. Then they were weighed and ground to pass a 1-mm sieve. Calcium, nitrogen, total and phytate phosphorus contents were determined by the same chemical methods used in diet analysis. Retention was calculated as follows:

% Retention = (consumed – excreted) × 100/consumed

Statistics. Three-way ANOVA was used to determine main effects (cereals, phytase and calcium levels) and their interactions using General Linear Models procedure. Mean differences were separated by Tukey's test. The level of significance was set at $P < 0.05$. The data were analysed by the General Linear Models procedure of the SAS 8.00 (SAS Institute, 1999).

RESULTS

Nutrient excretion

Average nitrogen, calcium, total and phytate phosphorus excretions in the 2nd and 3rd week of trial are listed in Table 2.

Main effects of cereals on nitrogen excretion were observed in both weeks of trial ($P < 0.0001$ and 0.0018, respectively). In both weeks the highest nitrogen excretion was observed in broilers fed wheat based diets and lowest in those fed barley based diets. In the 3rd week broilers that were fed maize, wheat and triticale based diets had significantly higher nitrogen excretion in comparison with those fed barley based diets.

Main effects of cereals and dietary Ca level on Ca excretion were observed in both weeks. In the 2nd week the highest Ca excretion was observed in broilers fed maize and the lowest in those fed barley based diets. Ca excretion of broilers fed wheat and triticale based diets was not significantly different from either of them. In the 3rd week Ca excretion of broilers fed maize, wheat and triticale was signifi-

Table 1. Composition of experimental diets (%)

Ingredient	Ca (%)							
	0.6	1.0	0.6	1.0	0.6	1.0	0.6	1.0
Ground maize	57.23	55.20						
Wheat			65.94	63.74				
Barley					62.13	61.00		
Triticale							59.80	57.70
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal	33.50	33.80	24.80	25.40	28.50	28.60	28.50	28.96
Oil	3.80	4.50	3.70	4.30	4.00	4.00	6.40	7.00
Calcium carbonate	0.07	1.10	0.19	1.21	0.14	1.17	0.12	1.14
Dicalcium phosphate	1.40	1.40	1.25	1.25	1.24	1.24	1.18	1.20
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
VAM PT[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.20	0.20	0.20	0.20	0.19	0.19	0.20	0.20
L-lysine			0.12	0.10				
Calculated analysis								
Crude protein (%)	21.06	21.06	21.09	21.07	21.08	21.01	21.05	21.04
Crude fat (%)	6.28	6.92	5.36	5.93	5.65	5.64	7.90	8.00
ME (kcal/kg)	3.003	3.007	3.003	3.001	3.024	2.992	2.998	3.002
Ca (%)	0.6	1.0	0.6	1.0	0.6	1.0	0.6	1.0
P_{tot} (%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
P_{ava} (%)[2]	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
P_{phy} (%)	0.27	0.27	0.26	0.26	0.27	0.27	0.24	0.24

ME – metabolic energy, P_{tot} – total phosphorus, P_{ava} – available phosphorus, P_{phy} – phytate phosphorus

[1]Vitamin and trace mineral premix provided (kg): vitamin A 13 500 i.u., vitamin D_3 2 000 i.u., vitamin E 30 mg, vitamin K_3 2 mg, vitamin B_1 1 mg, vitamin B_2 6 mg, niacin 30 mg, pantothenic acid 12 mg, vitamin B_6 3 mg, vitamin B_{12} 10 µg, biotin 0.1 mg, choline chloride 500 mg, Fe 50 mg, Cu 8 mg, Mn 80 mg, Zn 60 mg, I 0.5 mg, Co 0.2 mg, Se 0.15 mg, sodium monensin 100 mg and flavophospholipol 3 mg

[2] Based on analysed values of total and phytate phosphorus content in experimental diets

cantly higher than in those fed barley based diets. In both weeks significantly higher Ca excretion was observed in broilers fed diets containing 1.0% Ca ($P < 0.0001$). Several significant interactions were observed. In the 2nd week a cereal × Ca interaction was significant ($P < 0.0022$). Ca excretion of broilers fed diets containing 0.6% Ca decreased in the order wheat > barley > maize > triticale, while in those fed diets containing 1.0% Ca in the order maize > triticale > wheat ≈ barley. In both weeks a Ca × phytase interaction was significant ($P < 0.0191$ and 0.0125, respectively). In the 2nd week Ca excretion of broilers fed diets containing 0.6% Ca decreased while in those fed diets containing 1.0% Ca it increased with increasing supplemental phytase. In the 3rd week Ca excretion of broilers fed diets containing 0.6% Ca decreased in the order 500 > 1 000 ≈ 0 PU/kg, while in those fed diets containing 1.0% Ca it increased again with increasing supplemental phytase.

Total phosphorus excretion was influenced by phytase supplementation in the 3rd week ($P < 0.0483$),

Table 2. Average excretion of nitrogen, calcium, total (P_{tot}) and phytate phosphorus (P_{phy}) in the 2nd and 3rd week of study (in %)

	Df	P > F							
		N		Ca		P_{tot}		P_{phy}	
		2nd week	3rd week	2nd week	3rd week	2nd week	3rd week	2nd week	3rd week
Phytase	2	0.5762^{NS}	0.0618^{NS}	0.3594^{NS}	0.0590^{NS}	0.6795^{NS}	0.0483	0.0239	0.0527^{NS}
Cereal	3	< 0.0001	0.0018	0.0433	0.0010	0.0026	0.0001	0.0041	0.0317
Ca	1	0.1539^{NS}	0.8719^{NS}	< 0.0001	< 0.0001	0.0250	0.3539^{NS}	< 0.0001	< 0.0001
Phytase × cereal	6	0.7628^{NS}	0.5698^{NS}	0.1140^{NS}	0.0717^{NS}	0.8371^{NS}	0.0136	0.5575^{NS}	0.3160^{NS}
Phytase × Ca	2	0.2308^{NS}	0.3649^{NS}	0.0191	0.0125	0.7401^{NS}	0.1805^{NS}	0.4200^{NS}	0.6394^{NS}
Cereal × Ca	3	0.5039^{NS}	0.0579^{NS}	0.0022	0.0995^{NS}	0.6794^{NS}	0.1393^{NS}	0.4017^{NS}	0.0843^{NS}
Main effects									
Phytase (PU/kg)	0	5.02	4.87	1.30	1.41	1.45	1.52^{a}	0.59^{a}	0.57
	500	5.08	5.02	1.34	1.46	1.47	1.56^{b}	0.48^{ab}	0.51
	1 000	5.07	5.10	1.34	1.47	1.48	1.57^{b}	0.46^{b}	0.44
Cereal	maize	5.09^{a}	5.16^{a}	1.41^{a}	1.45^{a}	1.46^{a}	1.56^{a}	0.60^{a}	0.56^{ab}
	wheat	5.36^{b}	5.16^{a}	1.34^{ab}	1.53^{a}	1.54^{a}	1.64^{b}	0.59^{a}	0.57^{a}
	triticale	5.26^{ab}	5.09^{a}	1.29^{ab}	1.47^{a}	1.55^{a}	1.65^{b}	0.51^{a}	0.50^{ab}
	barley	4.54^{c}	4.58^{b}	1.27^{b}	1.32^{b}	1.32^{b}	1.35^{c}	0.35^{b}	0.39^{b}
Ca (%)	0.6	5.02	4.99	0.83^{a}	0.85^{a}	1.50^{a}	1.54	0.36^{a}	0.33^{a}
	1.0	5.10	5.00	1.82^{b}	2.04^{b}	1.43^{b}	1.56	0.66^{b}	0.68^{b}

Df = degree of freedom; NS = not significant; means with the same letter are not significantly different

different cereals in both weeks ($P < 0.026$ and 0.0001, respectively) and dietary Ca level in the 2nd week ($P < 0.0250$). Supplemental phytase significantly increased total phosphorus excretion in the 3rd week. Broilers fed maize, wheat and triticale based diets had significantly higher total phosphorus excretion than those fed barley based diets in the 2nd week of study. In the 3rd week broilers fed wheat and triticale based diets had significantly higher total phosphorus excretion than those fed maize based diets, which in turn had significantly higher excretion than broilers fed barley based diets. Total phosphorus excretion in the 2nd week of trial was significantly higher in broilers fed diets containing 0.6% Ca. A significant phytase × cereal interaction was observed in the 3rd week ($P < 0.0136$). In broilers fed maize total phosphorus excretion decreased in the order 500 > 0 > 1 000 PU/kg, in those fed wheat in the order 500 > 1 000 PU/kg > 0, in those fed triticale 1 000 > 0 > 500 PU/kg and in those fed barley 1 000 > 500 > 0 PU/kg.

Main effects of phytase supplement on phytate phosphorus excretion were observed only in the 2nd week ($P < 0.0239$), while those of cereals ($P < 0.0041$ and 0.0317) and dietary Ca level ($P < 0.0001$) in both weeks. Supplemental phytase significantly reduced phytate phosphorus excretion. In the 2nd week phytate phosphorus excretion of broilers fed maize, wheat and triticale based diets was significantly higher than of those fed barley based diets. In the 3rd week broilers fed wheat had significantly higher phytate phosphorus excretion than broilers fed barley, while phytate phosphorus excretions of broilers fed wheat and triticale based diets were not significantly different from each other and from the other two cereals. In both weeks phytate phosphorus excretion increased with increasing dietary Ca level.

Nutrient retention

Average nitrogen, calcium, total and phytate phosphorus retention in the 2nd and 3rd week of trial is listed in Table 3.

Main effects of cereals ($P < 0.0101$) and Ca ($P < 0.0327$) as well as their interaction ($P < 0.0494$) on nitrogen retention were observed only in the 2nd week. The significantly highest nitrogen retention was observed in broilers fed maize based diets. No significant differences were observed between broilers fed wheat, triticale and barley based diets. Nitrogen retention significantly increased with increasing dietary Ca level. The analysis of cereal × Ca interaction showed that nitrogen retention increased with Ca dietary level in broilers fed wheat and barley based diets but it almost did not change in broilers fed maize based diets, and decreased in broilers fed triticale based diets.

Cereals ($P < 0.0169$ and 0.0436) and dietary Ca levels ($P < 0.0001$) significantly influenced Ca retention in both weeks of trial. In the 2nd week Ca retention was higher in birds fed maize, wheat and triticale based diets, but differences in the 3rd week could not be detected by Tukey's test. An increasing Ca dietary level decreases calcium retention. In the 2nd week phytase × Ca and cereal × Ca interactions were observed. Ca retention of broilers fed diets containing 0.6% Ca decreased in the order 1 000 ≈ 500 > 0 PU/kg and wheat > maize ≈ triticale ≈ barley, while in those fed diets containing 1.0% Ca in the order 0 > 500 ≈ 1 000 PU/kg and maize ≈ triticale > wheat ≈ barley.

Supplemental phytase ($P < 0.0146$) and calcium ($P < 0.0010$) main effects as well as cereals × Ca interaction ($P < 0.0245$) on total phosphorus retention were observed only in the 2nd week. Total phosphorus retention increased with increasing

Table 3. Average retention of nitrogen, calcium, total (P_{tot}) and phytate phosphorus (P_{phy}) in the 2nd and 3rd week of study (in %)

	Df	P > F							
		N		Ca		P_{tot}		P_{phy}	
		2nd week	3rd week	2nd week	3rd week	2nd week	3rd week	2nd week	3rd week
Phytase	2	0.0694^{NS}	0.2195^{NS}	0.1780^{NS}	0.5352^{NS}	0.0146	0.8910^{NS}	0.0442	0.2171^{NS}
Cereal	3	0.0101	0.0624^{NS}	0.0169	0.0436	0.0654^{NS}	0.0647^{NS}	0.0388	0.3255^{NS}
Ca	1	0.0327	0.5683^{NS}	< 0.0001	<0.0001	0.0010	0.4333^{NS}	0.0002	0.0002
Phytase × cereal	6	0.0857^{NS}	0.5009^{NS}	0.0935^{NS}	0.1693^{NS}	0.1765^{NS}	0.2163^{NS}	0.4031^{NS}	0.6885^{NS}
Phytase × Ca	2	0.5188^{NS}	0.3855^{NS}	0.0091	0.7064^{NS}	0.1059^{NS}	0.8755^{NS}	0.2407^{NS}	0.6972^{NS}
Cereal × Ca	3	0.0494	0.2526^{NS}	0.0022	0.1757^{NS}	0.0245	0.2571^{NS}	0.1311^{NS}	0.0679^{NS}
Main effects									
Phytase (PU/kg)	0	63.2	68.0	61.7	63.5	45.8^{a}	49.1	57.2^{a}	62.0
	500	63.7	66.2	63.8	63.4	49.5^{ab}	49.8	64.1^{ab}	64.1
	1 000	65.7	66.0	64.4	62.3	51.8^{b}	49.8	67.6^{b}	69.2
Cereal	maize	67.5^{a}	68.5	65.1^{a}	65.7	52.2	50.8	56.1^{a}	60.8
	wheat	63.8^{b}	68.2	64.5^{a}	63.5	49.3	51.1	60.6^{ab}	63.7
	triticale	63.1^{b}	65.1	64.7^{a}	61.5	47.1	45.4	64.7^{ab}	66.8
	barley	62.4^{b}	65.1	58.9^{b}	61.5	47.5	51.0	70.3^{b}	69.1
Ca (%)	0.6	63.2^{a}	66.4	68.0^{a}	70.4^{a}	45.6^{a}	49.0	73.6^{a}	77.7^{a}
	1.0	65.2^{b}	67.0	58.6^{b}	55.7^{b}	52.5^{b}	50.1	52.2^{b}	52.6^{b}

Df = degree of freedom; NS = not significant; means with the same letter are not significantly different dietary Ca level and phytase supplementation. 1 000 PU/kg produced a significant effect. Total phosphorus retention in broilers fed diets containing 0.6% Ca decreased in the order maize > triticale > wheat ≈ barley, and in those fed diets with 1.0% Ca in the order wheat > maize ≈ barley > triticale.

All three main effects on phytate phosphorus retention were observed in the 2nd week and only dietary Ca level in the 3rd. Increasing supplemental phytase increased phytate phosphorus retention ($P < 0.0442$). The effect of 500 PU/kg was not significantly different from the effects of 0 and 1 000 PU/kg, which were significantly different from each other. The highest phytate phosphorus retention was observed in broilers fed barley based diets and the lowest in those fed maize based diets. Phytate phosphorus retentions of broilers fed wheat and triticale based diets were not significantly different from each other and from those of broilers fed barley and maize. In both weeks higher phytate phosphorus retention was observed in broilers fed diets containing 0.6% Ca. No significant interactions were observed indicating that the main effects were independent.

DISCUSSION

Intensive poultry production has raised the problem of environmental pollution, with nitrogen and phosphorus from excreta being recognized as main pollutants. Different dietary strategies have been employed in order to reduce environmental pollution, bearing in mind that costs of production should remain at least at the same level. The influence of dietary treatment on the excretion of possibly harmful substances that were not adequately digested and absorbed can be reduced by making balance between nutrient intake and excretion.

Sebastian *et al.* (1996, 1997), Simons *et al.* (1990), Broz *et al.* (1994) and Yi *et al.* (1996) showed that phytase addition to male broiler diets resulted in increased phosphorus, nitrogen and calcium retention. However, this effect was not observed in female broilers.

The results obtained in this research are in partial agreement with literature. The increasing phytase level resulted in a reduction in phytate phosphorus excretion and in an increase in its retention (differences were not always significant). This beneficial influence of phytase on excretion and retention was not so clearly observed in the case of total phosphorus because the diets contained recommended levels of total phosphorus.

The significance of calcium and phytate interplay was also shown in this study. Retention of both total and phytate phosphorus decreased while excretion increased with increasing calcium dietary levels.

According to Sebastian (1996b) phosphorus retention decreases with increasing calcium dietary levels due to: (*i*) precipitation of phytate through the Ca-phytate complex formation, (*ii*) increased intestinal pH, caused by Ca, which reduces mineral solubility, (*iii*) direct effect of Ca on phytate by competing for the active site of phytase. In this experiment the retention of phytate phosphorus, but not of total phosphorus, decreased with increasing calcium dietary levels.

These results indicate that phytate phosphorus from the diets could be made almost completely available if the diet contained appropriate calcium levels, Ca : P_{tot} ratio and phytase level. In this case phosphorus, and possibly nitrogen excretion into the environment could be reduced to a minimum.

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