Effects of supplemental phytase on the mineral content in tibia of 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 cereal-soybean meal based diets on the mineral content in tibia (Ca, P, Fe, Mg, Cd, Zn). 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). Supplemental phytase increased the mineral levels in tibia. A statistically significant increase was observed for Fe ($P < 0.024$) and Mg ($P < 0.024$), but not for Ca, P, Cd and Zn contents in tibia. In the case of Fe and Mg contents significant cereal × phytase interactions were also observed ($P < 0.036$ and 0.002, respectively). Cereals influenced the contents of Fe ($P < 0.0001$), Mg ($P < 0.0001$), Cd ($P < 0.0001$) and Zn ($P < 0.003$) while dietary calcium levels affected only calcium content ($P < 0.008$).

Keywords: maize; wheat; triticale; barley; phytase; mineral content in tibia; calcium; phosphorus; iron; magnesium; cadmium; zinc

Phosphorus is one of the main essential elements involved in metabolic processes. Almost 80% of phosphorus in organisms is involved in the formation of mineralized tissues.

The strength of bones, especially those in legs, can have a significant influence on the quality of produced poultry meat. Among others, for the normal bone growth it is necessary that poultry diets contain required amounts of phosphorus and other important minerals (calcium, magnesium). Almost two thirds of total phosphorus in poultry diets are in the form of phytate phosphorus, which is unavailable to the poultry due to the low activity of phytase present in their digestive tract. In addition, phytate forms complexes with proteins (Enzymes in Animal Nutrition, 1998) and starch (Thompson and Yoon, 1984; Thompson, 1988), reducing their solubility and digestibility in this way. Phytate also forms low-soluble complexes with bi- and trivalent minerals calcium, magnesium, iron and zinc (Enzymes in Animal Nutrition, 1998), lowering their bioavailability and influencing the bone formation process.

Phytase supplementation to the diets is one of the main nutritional approaches to overcome these problems. Numerous investigations with broilers fed maize-soybean meal based diets have shown that supplemental phytase has a beneficial influence on the bone mineralization process in general (Qian *et al.*, 1996; Sebastian *et al.*, 1996; Huyghebaert, 1996, 1997; De Bruyne and Zwart, 1998; Denbow *et al.*, 1998; Sohail and Roland, 1999). Tibia ash and mineral content, density, length and strength increased in broilers fed diets with supplemental phytase.

Investigations with broilers fed diets based on other cereals did not give any straightforward results. Zyla *et al.* (2000) showed that phytase supplementation to diets based on wheat had no significant effect on bone mineralization in broilers. However, Kiiskinen *et al.* (1994) reported that the addition of 1 000 phytase units/kg to broiler diets based on

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soybean meal and wheat, barley or oat, with low content of mineral phosphorus or without it, was sufficient for normal bone mineralization.

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 papers we showed that: (*i*) magnitude of the beneficial effect of phytase supplementation on broiler performance varied between different cereals and calcium levels, (*ii*) supplemental phytase reduced the excretion of phytate phosphorus and increased its retention, magnitude of the effect depended on calcium dietary level, (*iii*) tibia ash content was not influenced by supplemental phytase (Pintar *et al*., 2004, 2005). In this paper effects on the mineral content in tibia are presented.

MATERIAL AND METHODS

Materials and manipulation. Two hundred forty-five day-old commercial male broiler chickens (Ross) were randomly distributed in 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.

Diet ingredients were obtained in bulk. The Novo CT phytase (Novo Nordisk) was used. Both ingredients and diets were analysed for humidity, ash, protein, fat, fibre, calcium by usual methods (ISO 6496, 1999; ISO 5984, 2002; ISO 5983, 1997; ISO 6492, 1999; ISO 5498, 1999; ISO 6490–1, 1985; ISO 6491, 1998). Phytate phosphorus content was determined by Sooncharernying and Edwards (1993) procedure. Compositions of diets are given in Table 1.

Bone mineralization. At the termination of the study all birds were killed and left tibias of three birds per treatment were removed. Tibias were dried to constant weight at 105°C, defatted and then ashed in a muffle furnace at 550°C. Ash was dissolved in concentrated HCl for mineral determination. Calcium was determined by a titrimetric method (ISO 6490–1, 1985), phosphorus by a spectrometric method (ISO 6491, 1998) and iron, magnesium, cadmium and zinc were determined by AAS (AAS–3 Carl Zeiss spectrometer).

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

RESULTS AND DISCUSSION

Calcium, phosphorus, iron, magnesium, cadmium and zinc contents of the tibia of broilers fed different diets after 21st day of age are listed in Table 2.

Numerous studies showed that tibia ash content increased with phytase addition (Sebastian *et al*., 1996; Qian *et al*., 1996; Huyghebaert, 1996, 1997; Sohail and Roland, 1999) when broilers were fed maize-soybean meal based diets. On the contrary, Perney *et al.* (1993) for the broilers fed maize-soybean meal based diets, and Zyla *et al*. (2000) for the broilers fed wheat-soybean meal based diets demonstrated that tibia ash content did not increase when phytase was added. Data for the tibia mineral content are contradictory. Some authors found out the variation of mineral content in the whole tibia (Sohail and Roland, 1999) while others stated that only mineral content in the tibia head (Sebastian *et al*., 1996) varied with phytase supplementation. It seems that the resulting phytase influence is more pronounced in older broilers.

The increasing Ca dietary level significantly increased Ca content ($P < 0.0081$). Ca content increased with increasing supplemental phytase but the effect was not statistically significant.

No main effects on phosphorus content were observed, which is probably due to the fact that all diets contained recommended phosphorus levels.

Main effects of phytase ($P < 0.0242$) and cereals ($P < 0.0001$), as well as phytase × cereal interactions ($P < 0.0361$) on iron content were observed. Iron content in the tibia of broilers fed diets containing 1 000 PU/kg was significantly higher than in those fed diets without phytase supplement, but the iron content in broilers fed diets containing 500 PU/kg was not significantly different from any of them. Broilers fed wheat, triticale and barley based diets had a significantly higher iron content in tibia than those fed maize based diet. Iron content in the tibias of broilers fed maize, wheat and barley based diets increased with increasing phytase content

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 – metabolizable 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, monensin sodium 100 mg and flavophospholipol 3 mg

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

while in those of broilers fed triticale it decreased in the order 500 > 0 > 1 000 PU/kg.

Magnesium content was affected by main effects of phytase ($P < 0.0240$) and cereals ($P < 0.0001$), as well as phytase × cereal ($P < 0.0023$) and cereal × Ca ($P < 0.0093$) interactions. The increasing phytase supplement increased magnesium content. Magnesium content in broilers fed diets containing 1 000 PU/kg was significantly higher than in those fed diets without phytase supplement, but that of broilers fed diets containing 500 PU/kg was not significantly different from any of them. Broilers fed wheat, triticale and barley based diets had a significantly higher magnesium content in tibia than those fed maize based diet. Increasing supplemental phytase resulted in increasing magnesium content in the tibia of broilers fed maize and barley based diets. In broilers fed wheat based diets magnesium content decreased in the order 0 > 500 ≈ 1 000 PU/kg while in those fed triticale based diets in the order 500 > 0 > 1 000 PU/kg. Magnesium content in the tibia of broilers fed wheat, barley and

Table 2. Calcium, phosphate, iron, magnesium, cadmium and zinc contents in tibia

	Df	P > F					
		Ca	P	Fe	Mg	Cd	Zn
Phytase	2	0.0574[NS]	0.9958[NS]	0.0242	0.0240	0.0732[NS]	0.9751[NS]
Cereal	3	0.1418[NS]	0.3193[NS]	< 0.0001	< 0.0001	< 0.0001	0.0031
Ca	1	0.0081	0.0709[NS]	0.8396[NS]	0.4167[NS]	0.4804[NS]	0.9650[NS]
Phytase × cereal	6	0.4243[NS]	0.5513[NS]	0.0361	0.0023	0.2706[NS]	0.4074[NS]
Phytase × Ca	2	0.8877[NS]	0.6705[NS]	0.0933[NS]	0.1131[NS]	0.0958[NS]	0.5009[NS]
Cereal × Ca	3	0.1469[NS]	0.2865[NS]	0.6932[NS]	0.0093	< 0.0001	0.0041
		%		μg/g dm	mg/g dm	μg/g dm	
Main effects							
Phytase (PU/kg)	0	35.7	16.4	103.1[a]	3.5[a]	3.2	329.0
	500	35.6	16.5	114.8[ab]	3.8[ab]	2.9	331.2
	1 000	35.9	16.5	120.1[b]	3.9[b]	3.0	331.6
Cereal	Maize	35.8	16.4	43.3[a]	3.0[a]	3.7[a]	296.5[b]
	Wheat	35.8	16.1	114.5[b]	4.0[b]	3.0[b]	345.3[a]
	Triticale	35.6	16.9	142.5[b]	4.0[b]	2.8[b]	335.4[ab]
	Barley	35.9	16.5	152.3[b]	4.3[b]	2.7[b]	345.3[a]
Ca (%)	0.6	35.6[a]	16.7	113.2	3.8	3.0	330.4
	1.0	35.9[b]	16.2	112.2	3.7	3.0	330.8

Df – degree of freedom, dm – dry matter

[NS]not significant; means with same letter are not significantly different

triticale based diet decreased while that of broilers fed maize based diet increased with increasing dietary calcium level.

These findings are in accordance with the literature data that shows that supplemental phytase increases the mineral content in tibia (Sohail and Roland, 1999). However, we were unable to find any literature data on the phytase influence on iron and magnesium content in broiler tibia. It is interesting to note that contents of both minerals were statistically higher in broilers fed wheat, triticale or barley based diets in comparison with those fed maize based diets. This could be ascribed, at least partially, to the higher phytase activity of wheat, barley and triticale in comparison with maize (Viveros *et al.*, 2000).

Main effect of cereal ($P < 0.0001$) on cadmium content was observed. Broilers fed wheat, triticale and barley based diets had a significantly lower cadmium content in tibia that those fed maize based diet. A significant cereal × Ca ($P < 0.0001$) interaction was also observed. Cadmium content in the tibia of broilers fed diets containing 0.6% Ca decreased in the order maize > wheat > triticale > barley while in those fed diets containing 1.0% Ca in the order maize > barley > wheat > triticale.

Zinc content was significantly affected by cereals ($P < 0.0031$). Broilers fed wheat and barley had a significantly higher content than those fed maize based diets. Zinc content in the tibia of broilers fed triticale was not significantly different from others. A significant cereal × Ca interaction was observed. Zinc content in the tibia of broilers fed diets containing 0.6% Ca decreased in the order barley > triticale > maize ≈ wheat while in those fed diets containing 1.0% Ca in the order wheat > triticale > barley > maize.

These findings are in contrast with findings of Mohanna and Nys (1999) and Zanini and Sazzad (1999) that in both 1 day old and 21 days old chickens supplemental phytase increases zinc content in tibia.

CONCLUSION

Supplemental phytase influences bone mineralization in broilers; although proven beneficial in general, the problem is not completely understood. Ambiguities still remain about the phytase influence on the content of essential minerals in bones. Available results point out that when addressing this problem, all experimental parameters should be taken into account in order to assess the role phytase plays in the mineralization process.

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