Effects of supplemental phytase on performance and tibia ash of broilers fed different cereals 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 broiler performance and tibia ash. 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). Supplemented phytase had beneficial effects on broiler performance. It significantly increased body weight gain ($P < 0.0003$) and feed consumption ($P < 0.0361$) by 6 and 7% in comparison with the control groups, respectively. No influence on feed conversion ratio and tibia ash was detected. Both body weight gain and feed intake were also significantly influenced by different cereals ($P < 0.0001$ and 0.0348, respectively). The increasing dietary calcium level resulted in a significant increase in body weight gain ($P < 0.0024$) and tibia ash ($P < 0.0016$). Effects of 500 and 1 000 PU/kg were not statistically different between themselves.

Keywords: phytase; maize; wheat; triticale; barley; body weight gain; feed consumption; feed conversion ratio; tibia ash

Phosphorus is an essential mineral in growth and development of poultry.

Poultry diets are usually based on cereals in which 50–70% of total phosphorous is present in the form of phytate (Ravindran *et al*., 1995). This phosphorous is unavailable to monogastric animals because they lack endogenous phytase. One way to solve this problem is the addition of inorganic phosphorus sources to diets. On the one hand, this approach increases the cost of production and on the other, it does not solve the problem of phosphorous excretion into the environment.

Supplementation of enzyme phytase to poultry diets has proven to be an efficient solution when addressing these problems. Numerous studies showed that supplemental phytase increased body weight gain – BWG (Qian *et al*., 1996a; Sebastian *et al*., 1996a; Yi *et al*., 1996), phosphorus bioavailability (Sebastian *et al*., 1996b; Windisch and Kirchgessner, 1996) as well as bioavailability of amino acids, Zn, Ca, Mg (Ravindran *et al*., 1999; Um and Paik, 1999), improved biomineralization (Sebastian *et al*., 1996a) and decreased phosphorus and nitrogen excretion (Yi *et al*., 1996; Edens *et al*., 1999).

Effect of phytase supplementation in maize-soybean meal based diets has been studied thoroughly, but investigations of diets based on other cereals are scarce. Zyla *et al*. (2000) showed that supplementation of 750 PU/kg to wheat based diet increased body weight gain, feed consumption and phosphorus retention. Supplementation of phytase (1 200 PU/kg) improved the digestibility of protein and amino acids in maize, sorghum and wheat based diets, but the magnitude of response varied between cereals (Ravindran *et al*., 1999). Normal broiler bone mineralization was supported by supplementing 1 000 PU/kg to diets based on wheat, barley, oat and soybean meal with low or zero inorganic phosphorus content (Kiiskinen *et al*., 1994).

The aim of this investigation was to determine and compare effects of phytase supplementation to diets based on maize, wheat, barley and triticale on broiler performance, nutrient retention and excretion as well as bone mineralization. In this paper

Supported by the Ministry of Science and Technology of the Republic Croatia (Grant No. 178881).

effects on bird performance and tibia ash 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 broilers were maintained in wire-floored pens with separated excreta trays, placed in an environmentally controlled room. The birds received constant electrical illumination.

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

Diet ingredients were obtained in bulk. 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 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. 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 obtained 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 phosphorous determination (ISO 6491, 1998).

Chemical compositions of feeds and experimental diets are given in Tables 1 and 2, respectively.

Broiler performance. Body weights were measured weekly and additionally at the first three days of the 2nd and 3rd week. Feed consumptions were determined in the first three days of the 2nd and 3rd week of study. Feed conversion was then calculated as a feed consumption to body weight gain (BUG) ratio. Only the results of the 3rd week of trial are presented.

Tibia ash. 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.

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

RESULTS

Statistical analyses of the effects of phytase, different cereal and calcium contents on average body

Table 1. Composition of feeds (in %)

Ingredient	Moisture	Ash	Crude protein	Crude fat	Crude fibre	Ca	P_{tot}	P_{phy}
Corn	14.87	0.89	6.93	2.92	2.66	0.08	0.28	0.21
Wheat	12.65	1.56	11.71	1.53	2.54	0.11	0.34	0.26
Barley	11.01	1.62	9.98	1.52	2.55	0.13	0.32	0.24
Triticale	13.55	2.03	10.31	1.36	3.39	0.17	0.35	0.24
Soybean meal	12.22	5.95	44.23	1.48	5.78	0.41	0.74	0.49
Fish meal	7.00	11.50	72.00	9.30		2.10	1.83	
Dicalcium phosphate	3.31					23.10	19.16	
Calcium carbonate	0.003					39.06		

P_{tot} = total phosphorus, P_{phy} = phytate phosphorus

Table 2. 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_{phyt} (%)	0.27	0.27	0.26	0.26	0.27	0.27	0.24	0.24
Analysed values								
Crude protein (%)	20.85	21.10	21.08	20.80	20.75	20.99	21.19	20.63
Crude fat (%)	6.49	6.76	5.40	5.87	5.51	5.52	8.14	9.27
Ca (%)	0.64	1.01	0.66	1.02	0.63	1.02	0.65	1.02
P_{tot} (%)	0.67	0.64	0.69	0.69	0.67	0.71	0.69	0.72
P_{phy} (%)	0.32	0.28	0.34	0.32	0.34	0.35	0.32	0.33

[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, flavophospholipol 3 mg

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

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

weight gain, feed consumption, feed conversion and tibia ash in the period 0–21 days are summarised in Table 3.

Body weight gain

All three main effects had a significant influence on body weight gains of broilers fed different diets. Phytase supplementation caused a 6.0% increase in BWG in comparison with the control ($P < 0.0003$). But there was no significant difference between BWG of broilers fed diets containing 500 and 1 000 PU/kg. BWG of broilers fed maize and wheat based diets were significantly higher than those of broilers fed barley, which were significantly higher than BWG of broilers fed triticale based diets. The increased level of Ca (1.0%) improved BWG significantly ($P < 0.0024$).

Significant interactions between phytase and cereals ($P < 0.0016$) and between cereals and Ca ($P < 0.0016$) were observed, indicating that the effects of these factors are not independent. BWG of broilers fed wheat and triticale based diets increased with increasing phytase supplement, broilers fed barley based diet had the highest BWG when 500 PU/kg was supplemented to the diets, while BWG of broilers fed maize based diet decreased with increasing supplemental phytase. BWG of broilers fed maize, triticale and barley based diets increased with increasing Ca level, while those of broilers fed wheat based diets decreased.

Feed consumption

Feed consumption was significantly affected by phytase supplementation ($P < 0.0361$) and different cereals ($P < 0.0348$). Broilers fed diets containing 1 000 PU/kg had significantly higher feed consumption (7.0%) than those fed diets without supplemented phytase. But the feed consumption

Table 3. Effects of phytase, different cereals and calcium content on average body weight gain, feed consumption, feed conversion and tibia ash in the period 0–21 day

	Df	P > F			
		Body weight gain	Consumption	Conversion	Tibia ash
Phytase	2	0.0003	0.0361	0.4084^{NS}	0.7137^{NS}
Cereal	3	< 0.0001	0.0348	0.5062^{NS}	0.1317^{NS}
Ca	1	0.0024	0.0900^{NS}	0.8783^{NS}	0.0016
Phytase × cereal	6	0.0016	0.2868^{NS}	0.1059^{NS}	0.1293^{NS}
Phytase × Ca	2	0.0720^{NS}	0.7035^{NS}	0.6508^{NS}	0.2840^{NS}
Cereal × Ca	3	0.0016	0.2504^{NS}	0.6733^{NS}	0.0535^{NS}
		g	g	g : g	%
Main effects					
Phytase (PU/kg)	0	622.8^{a}	886.6^{a}	1.4	47.6
	500	660.6^{b}	913.6$^{a.b}$	1.4	48.3
	1 000	663.4^{b}	946.8^{b}	1.4	47.9
Cereal	Corn	665.2^{a}	951.8^{a}	1.4	47.9
	Wheat	677.8^{a}	932.2^{ab}	1.4	47.7
	Triticale	610.1^{b}	874.7^{b}	1.4	46.8
	Barley	642.5^{c}	903.9^{ab}	1.4	49.4
Ca (%)	0.6	638.6^{a}	901.4	1.4	46.2^{a}
	1.0	659.2^{b}	929.9	1.4	49.6^{b}

^{NS} not significant, means with same letter are not significantly different

Df = degree of freedom

of broilers fed diets supplemented with 500 PU/kg was not significantly different from that of broilers fed diets without phytase and diets with 1 000 PU per kg. The highest feed consumption was observed in broilers fed maize based diets, and the lowest in broilers fed triticale based diets. Feed intakes of broilers fed wheat and barley based diets were not significantly different between them and from feed intakes of broilers fed both maize and triticale based diets. No significant interactions were observed indicating that the main effects are independent.

Feed conversion

There were no significant differences in feed conversion between broilers fed different diets.

Tibia ash

The main effect of dietary Ca level on tibia ash of broilers fed different diets was the only one observed. As expected, broilers fed diets containing a higher Ca level had significantly higher tibia ash.

DISCUSSION

Beneficial effects of supplemented phytase on BWG and feed consumption of the broilers fed diets based on different cereals and soya, obtained in this research, are in accordance with numerous studies. Simons *et al*. (1990) and Sebastian *et al*. (1996a,b) reported increased BWG and feed intake of broilers fed maize-soybeans based diets upon the addition of supplemental phytase. Yi *et al*. (1996) achieved 11 to 22% increase in BWG and 6–25% increase in feed consumption, of both male and female chickens, when phytase was supplemented to the diets. Windisch and Kirchgessner (1996) found that phytase addition to broiler diets based on maize and wheat also increased bird performance. The magnitude of the supplemental phytase effect in this study depended on the cereal on which the diet was based, similar to the findings of Ravindran *et al*. (1999).

There were no significant differences in feed conversion between different groups, similar to the results obtained by Edwards (1993), Broz *et al*. (1994), Sebastian *et al*. (1996a), Yi *et al*. (1996), Huyghebaert (1996, 1997) and Harter-Denis (1999). On the contrary, Ravindran *et al*. (1995) reported better feed conversion when 800 PU/kg were added to the diets. Sebastian *et al*. (1996a) also established that better feed conversion could be obtained by phytase supplementation to the diets with low Ca content.

Supplemental phytase did not influence tibia ash, which is in accordance with the results of Perney *et al*. (1993), but in contrast to the results obtained by Sebastian *et al*. (1996a), Sohail and Roland (1999), Qian *et al*. (1996b), Huyghebaert (1996, 1997) and Broz *et al*. (1994). These results were obtained for maize-soybeans based diets. Zyla *et al.* (2000) showed that phytase addition to wheat-soya based diets had no significant effect on bone mineralization. This different observation can be explained by Underwood's finding (1981) that the Ca to nonphytate phosphorus ratio beyond 2 : 1 reduces bioavailability of Ca and P due to the formation of insoluble calcium-phosphate complex in the chicken gut.

Several explanations of better bird performance due to the phytase supplementation to the diets have been given. Simons *et al*. (1990) ascribed this effect to the release of minerals from their complex with phytate and/or utilisation of inositol. Other possible reasons are an increase in starch digestibility (Knuckles and Betschart, 1987) and bioavailability of proteins (Smith and Rackis, 1957).

In conclusion, we have shown beneficial effects on broiler performance of phytase supplemented to diets based on different cereals and soya, but the difference between the effects of different supplemented levels is not clear. In their recent study Shirley and Edwards (2003) showed that maximum of broiler performance was achieved when 12 000 PU/kg wa supplemented to diets. Both these results indicate the need to re-evaluate current recommendations for phytase supplementation taking into account on which cereal the diet is based.

Acknowledgment

We are grateful to prof. Željko Berić for useful discussions and to Mrs. Ivana Brnić and Jelena Kutnjak for help with chemical analyses.

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Received: 04–02–13
Accepted after corrections: 04–10–07

ABSTRAKT

Vliv doplňkové fytázy na užitkovost a obsah popelovin v holenní kosti brojlerů dostávajících krmné směsi na základě různých obilovin

Uskutečnili jsme 21denní pokus s jednodenními brojlery, abychom zhodnotili vliv doplňku fytázy do krmných směsí sestavených na základě různých obilovin a extrahovaného sójového šrotu na užitkovost brojlerů a obsah popelovin v holenní kosti. Krmné směsi obsahovaly 4 různé druhy obilovin (kukuřici, pšenici, triticale a ječmen), 2 hladiny vápníku (0,6 a 1,0 %) a 3 hladiny doplňkové fytázy (0, 500 a 1 000 PU/kg). Doplněk fytázy měl příznivé účinky na užitkovost brojlerů. Ve srovnání s kontrolními skupinami došlo k významnému zvýšení přírůstku tělesné hmotnosti ($P < 0,0003$) a spotřeby krmiva ($P < 0,0361$) o 6% resp. o 7%. Nezaznamenali jsme žádný vliv na konverzi krmiva ani na obsah popelovin v holenní kosti. Jednotlivé druhy obilovin také významně ovlivnily přírůstek tělesné hmotnosti a příjem krmiva ($P < 0,0001$ resp. $P < 0,0348$). Vyšší hladina vápníku v krmné směsi vedla k významnému zvýšení přírůstku tělesné hmotnosti ($P < 0,0024$) a obsahu popelovin v holenní kosti ($P < 0,0016$). Účinky hladiny 500 a 1 000 PU/kg nebyly statisticky rozdílné.

Klíčová slova: fytáza; kukuřice; pšenice; triticale; ječmen; přírůstek tělesné hmotnosti; spotřeba krmiva; konverze krmiva; obsah popelovin v holenní kosti

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