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EFFECT OF LOW- AND HIGH PHYTASE BARLEY ON PHOSPHORUS RETENTION AND BONE MINERAL COMPOSITION IN BROILERS

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Abstract

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The effects of low-phosphorus diets with two levels of phytase were examined in broiler chickens using eight replicates per treatment. The diets contained 40 % of spring barley with either 201 or 305 phytase activity units per kg. Chromic oxide was included in the diets as an indigestible marker. Excreta were collected during four consecutive three-day balance periods from the 12th to the 23rd day of age. No difference was observed for body weight gain between the dietary treatment groups but feed conversion ratio was better ($P < 0.05$) when higher phytase barley was used. In spite of the higher dietary level of phosphorus, the coefficient of apparent P retention was higher ($P < 0.01$) in higher than in lower phytase group. The retention of phosphorus increased ($P < 0.01$) with time of feeding in both low-phosphorus dietary groups. Differences between treatments in concentration of calcium, phosphorus, magnesium, sodium and potassium in defatted tibia were not significant ($P > 0.05$).

Keywords: plant phytase; low-phosphorus diet; P-retention; chicken

INTRODUCTION

Phytate, i. e. myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate serves as a storage form of phosphorus (P) in plant seeds (Reddy *et al.*, 1982). About 70 % of the P in barley is bound as phytate (Lott *et al.*, 2000).

Phytase is a naturally occurring enzyme that can degrade phytate to yield inositol and P (Liu *et al.*, 1998). One unit (1 U) of phytase activity is defined as the amount of enzyme which releases 1 µmol of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37 °C (ISO, 2009). Feedstuffs of plant origin contain small quantities of their own endo-phytases which can hydrolyze only a small part of total plant phosphorus. As poultry do not synthesize phytase (Nelson, 1967), much of the phytate P present in the diet is passed out into the excreta. Thus the requirement of animals for P must be met either by additional mineral P sources or by supplementation of industrially produced microbial phytase. Denbow *et al.* (1995) studied the supplements of phytase to broiler diets containing different levels of P. They observed improved body weight gain ($P < 0.01$) at all P levels, but the magnitude of response was greatest at low dietary level. Feed efficiency was unaffected by phytase addition.

The aim of the present experiment was to study the effect of low-P barley-soyabean meal-maize diets contaning two different cultivars of barley with different content of phytase activity on growth rate, feed efficiency and phosphorus retention in broiler chickens.

MATERIALS AND METHODS

The effects of phytase content and time of feeding upon the apparent P retention of diet were investigated in balance experiments with chickens during the fattening from the 12th to the 23rd day of age. The bone mineral composition of birds was also studied.

Animals and Procedures

The animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno. Eight day-old male Ross 308 broiler chickens were distributed according to body mass into two dietary treatments with eight replicates per treatment. There were 8 chickens per replicate pen. The animals were kept in balance cages (850 × 1000 mm) in an air-conditioned room. Heating and lighting programmes were in accordance with the Ross Broiler Management Handbook (2014). The birds were adapted to cages and experimental diets for 3 days. On day 12, four subsequent three-day balance periods started, during which excreta were collected daily. In each period, individual weight gains and feed consumption per pen were recorded. The coefficients of apparent P retention were estimated using the chromic oxide indicator method. Using this method, individual balance periods can follow subsequently without periods of starvation. Apparent phosphorus retention was calculated by the following formula

$$P\ retention(\%) = 100 - \frac{Cr_2O_{3,diet} \times P_{exc}}{Cr_2O_{3,exc} \times P_{diet}} \times 100$$

where P_{diet} is the dietary concentration of phosphorus and P_{exc} is concentration of phosphorus in excreta. Appropriate corrections were made for differences in moisture content.

On day 23, ten chickens from each treatment were randomly chosen and euthanized by cervical dislocation. The tibias were excised and defatted in petrolether. The central parts of tibias were used for the analysis of mineral concentrations.

Experimental Diets

During the whole experiment, all chickens were fed on a non-pelleted grower diet (Tab. I) containing 13.20 MJ nitrogen-corrected metabolisable energy and 210 g crude protein per kg. Except for phosphorus, two isocaloric barley-soybean meal-maize diets (40–31–15 %) were formulated to be in line with the Ross Nutrition Supplement (2009). To enable sensitive detection of changes in

I: *Composition of basal diet (as fed basis)*

Ingredients, %	
Barley	40.00
Soyabean meal 48 % CP	31.40
Maize	15.00
Soybean oil	9.90
Calcium carbonate	1.97
Monocalcium phosphate	0.33
Sodium chloride	0.33
DL-Methionine 99 %	0.27
L-Lysine.HCl 78 % Lys	0.13
L-Threonine 98 %	0.07
Supplementary premix[1)]	0.30
Chromic oxide	0.30
Calculated composition	
AME_n (MJ/kg)	13.20
Crude protein, %	21.00
SID Lysine, %	1.10
SID Met+Cys, %	0.84
SID Threonine, %	0.73
SID Valine, %	0.84
SID Isoleucine, %	0.75
Ca, %	0.90
Available P, %	0.20

[1] The premix supplied (mg/kg diet): retinyl acetate 4.13; cholecalciferol 0.128; DL-α-tocopherol acetate 56; menadione 3; thiamine 3; riboflavin 6; pyridoxine 4.1; hydroxycobalamine 0.015; niacin amide 50; pantothenic acid 18; biotin 0.2; folic acid 1.7; choline chloride 240; betaine 100; Narasin 70; copper 17; iron 50; zinc 80; manganese 100; iodine 1; cobalt 0.4; molybdenum 0.5; selenium 0.3; Endo-1,3(4)-beta-glucanase, Endo-1,4-beta-xylanase

phosphorus retention, the diets were calculated to contain 0.20 % of available phosphorus rather than the recommended value of 0.45 %.

The diets comprised 40 % of two different cultivars of spring barley, either with a low (LPB) or a high (HPB) phytase activity. Chromic oxide was added to the diets as a marker. The ingredient composition and calculated nutrient composition of diets are given in Tab. I. The diets were supplied *ad libitum*.

Chemical Analyses

The activity of barley phytase was determined according to ISO 30024:2009 (Animal feeding stuffs – Determination of phytase activity). The content of chromic oxide in food and freeze-dried excreta was estimated iodometrically (Mandel *et al.*, 1960). Phosphorus was estimated after wet mineralization by sulphuric acid and hydrogen peroxide spectrophotometrically as vanadate yellow using Unicam 8625 UV/VIS Spectrophotometer (LabX, Midland, ON, Canada) at a wavelength of 442 nm. Minerals in the tibia were estimated after decomposition in the microwave digestion system (MLS 1200 Mega; Milestone S.r.L., Sorisole, Italy) in the presence of nitric acid, hydrochloric acid and hydrogen peroxide on ICP-MS spectrometer (Agilent 7700x; Agilent Technologies, Tokyo, Japan). Measured isotopes were ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K and ^{44}Ca.

Statistical Analysis

Experimental data were analyzed as a completely randomized block design using ANOVA procedure of Statgraphics Plus package (Version 3.1, 1994). When a significant value for treatment effect ($P < 0.05$) was observed, the differences between means were assessed by Tukey HSD test. The experimental unit was a replicate pen.

RESULTS AND DISCUSSION

The activity of phytase in LPB was 201 U/kg, i. e. 34 % lower than that in HPB (305 U/kg). The intrinsic phytase activity in soybean meal used to prepare diets was not detected, and in maize it was 8 U/kg. The contents of total phosphorus were 4.56 and 4.72 g/kg in LPB and HPB diets, respectively.

The average body weights (mean ± standard error of the mean) of HPB and LPB chickens at 11 days of age were 252 ± 2.3 g and 257 ± 1.8 g, respectively. In contrast to the findings of Denbow *et al.* (1995), when animals were fed with LPB in this experiment, weight gains were slightly lower and the feed

II: *Effect of phytase on weight gains and feed conversion ratio*

Parameter	Diet	11–14 d	15–17 d	18–20 d	21–23 d	11–23 d
Weight gain (g)	HPB	108.4^{a}	140.2^{a}	159.4^{a}	158,9^{a}	566.8^{a}
	LPB	111.2^{a}	132.2^{a}	152.1^{a}	145.5^{b}	540.0^{a}
Feed conversion ratio	HPB	1.607^{a}	1.491^{a}	1.555^{a}	1.534^{a}	1.553^{a}
	LPB	1.586^{a}	1.578^{b}	1.624^{a}	1.686^{a}	1.647^{b}

HPB – High Phytase Barley
LPB – Low Phytase Barley
^{a,b} Means of HPB and LPB not sharing a common superscript were significantly different (Tukey HSD test, $P < 0.05$).

III: *Effect of phytase on apparent phosphorus retention at different age periods*

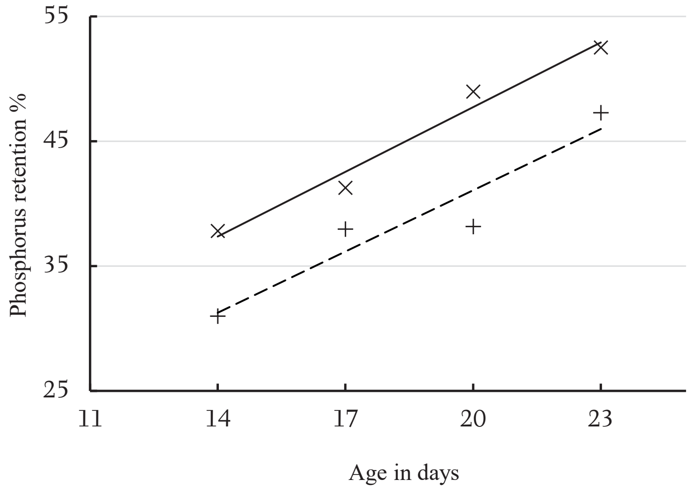
Parameter	Diet	12–14 d	15–17 d	18–20 d	21–23 d	12–23 d
P-retention %	HPB	37.81^{a}	41.28^{a}	48.98^{a}	52.51^{a}	45.14^{a}
	LPB	31.00^{b}	37.97^{b}	38.16^{b}	47.29^{b}	38.61^{b}

HPB – High Phytase Barley
LPB – Low Phytase Barley
^{a,b} Means of HPB and LPB not sharing a common superscript were significantly different (Tukey HSD test, $P < 0.05$).

IV: *Effect of phytase on tibia mineral density (g/kg)*[1]

Mineral	HPB	LPB
Phosphorus	82.19 ± 1.587	87.57 ± 2.424
Calcium	191.09 ± 4.085	196.15 ± 4.857
Magnesium	3.23 ± 0.074	3.21 ± 0.083
Sodium	3.16 ± 0.142	3.54 ± 0.163
Potassium	2.33 ± 0.139	2.58 ± 0.139

[1] In dry matter of defatted tibia (mean ± standard error of the mean)
HPB – High Phytase Barley
LPB – Low Phytase Barley



1: *Phosphorus retention from HPB (—x) and LPB (– – +) diets, respectively. The points are treatment means. Plotted from the equations $y_{HPB} = 13.19 + 1.727x$; $R^2 = 0.612$ and $y_{LPB} = 8.35 + 1.635x$; $R^2 = 0.629$*

conversion ratio was significantly ($P < 0.05$) worse when compared with HPB (Tab. II).

Juanpere *et al.* (2004) used a low-P diet with untreated (191 Phytase U/kg) or autoclaved barley in an experiment with broilers. Destruction of endogenous phytase had only a little negative influence ($P > 0.05$) on the total P retention coefficient and bone mineral density. Similar results with different intrinstic phytase activity of cereal grains (16–99 U/kg) were reported by Leytem *et al.* (2008). In contrast, the effect of endogenous phytase was significant ($P < 0.05$) in the present study. An average apparent phosphorus retention in the LPB and HPB diets was 38.6 % and 45.1 %, respectively. In spite of slightly higher level of phosphorus in the HPB group, the coefficient of apparent P retention was relatively 16.8 % and absolutely 6.5 % higher than in the LPB group (Tab. III).

The effect of duration of feeding on apparent phosphorus utilization was highly significant ($P < 0.01$). The feeding of the low-phosphorus diet resulted in a linear increase in P retention by 1.6–1.7 % for each day (Fig. 1). These results demonstrate that the organism adapts itself gradually to an insufficient supply of this essential mineral.

McDowell (1992) reported that the utilisation of P could be increased through active transport. In our previous experiment (Zelenka and Fajmonová, 2001) on chickens fed a diet with sufficient level of P, coefficients of apparent retention of P highly significantly decreased as the phosphorus requirement decreased with increasing age. On the other hand, the utilisation of P from a diet with a suboptimum level of P increased gradually with the time of feeding. Similarly Olukosi *et al.* (2007) found in a 21-day experiment with a diet marginally deficient in P that the apparent total tract retention of P increased ($P < 0.05$) with the time of feeding as the chicks grew from 1 to 3 weeks old. The ability of the chicks to retain P increased by 120 % from week 1 (15.7 %) to 2 (35.1 %) but the increase from week 2 to 3 (48.1 %) was only 40 %.

Concentration of macrominerals in dry matter of defatted tibia are shown in Tab. IV. Differences between HPB and LPB groups were not significant ($P > 0.05$).

CONCLUSION

The higher level of plant intrinsic phytase in barley is very efficacious for improving phosphorus utilization in fattening chickens. Retention of P from the diet with the suboptimum level of P increased gradually with the time of feeding.

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