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Digestibility of phosphorus in laying hens fed a wheat-maize-soyabean diet and the excreta phosphorus fractions*

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ABSTRACT

Availability of phytate phosphorus (P) was determined in ISA Brown hens 21 and 47 weeks old, fed a wheat-maize-soyabean diet, without a phytase supplement. The diet contained total and phytate P at 4.99 and 1.58 g/kg, respectively. The average body weight and hen-day egg production were 1.56 kg and 0.52, and 1.92 kg and 1.00 for younger and older hens, respectively. Phytate in the feed and faeces was determined by a capillary isotachophoretic method. Availability of phytate P and proportion of dietary Ca retained was non-significantly higher in older hens than in younger ones (47.3 and 56.7% vs 38.7 and 44.4%, respectively). Amount of P retained was similar in both groups (199 and 194 mg/day in younger and older birds, respectively). Amount of Ca retained was significantly higher in older than in younger hens (2.87 and 1.69 g/day, respectively; $P<0.05$). Dry matter of excreta of 47-week-old hens contained significantly ($P<0.05$) more total and phosphate P than excreta of 21-week-old hens (14.8 and 8.9 mg/g vs 12.3 and 5.9 mg/g dry matter, respectively). Phytate P represented 30.9% of excreta P in younger and 23.0% in older hens. Corresponding proportions of phosphate P were 48.0 and 60.1%. Differences between younger and older hens in proportions of P fractions were statistically significant ($P<0.05$). Excreta of 21-week-old hens and those of 47-week-old hens contained 78.1 and 63.0 mg of Ca/g dry matter, respectively ($P<0.05$). Thus, the age and different egg production significantly affect retention of Ca and excreta composition in laying hens. The effect of age on phytate availability was less pronounced.

KEY WORDS: laying hens, nutrition, phosphorus, phytate, age

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INTRODUCTION

Phytate is the major form of phosphorus (P) in ingredients of poultry diets. Availability of phytate P in poultry is rather low, varying from zero to over 50% (reviewed by Ravindran et al., 1995). The main dietary factors affecting phytate P availability in poultry are concentration of calcium (Ca) and activity of phytase present in feeds. Ca inhibits phytate hydrolysis and decreases P availability in poultry diets (Scheideler and Sell, 1987; Mohammed et al., 1991). Various feed components differ in endogenous phytase activity. In wheat and barley, the native phytase activity is high, whereas in maize and oil-seeds the phytase activity is low (Eeckhout and De Paepe, 1994). In most poultry diets native phytase activity is low, thus it is common practice to supplement diets with inorganic or organic P sources. The capacity of commercially available microbial phytase to increase total P digestibility has frequently been demonstrated (Selle and Ravindran, 2007). Inefficient P utilization in poultry results in increased P excretion, thus represents a hazard for quality of surface waters in areas of intensive animal production (Nahm, 2007).

As pointed out by Leytem et al. (2007), our understanding of the metabolism of phytate in monogastric animals is limited and information is needed on the P composition of faeces obtained from a wide range of animals and diets. More information is available on the metabolism of phytate P in chickens than in laying hens. Comparative studies showed that hens utilized phytate P more efficiently than chickens (Maddaiah et al., 1963; Nelson, 1976). It is generally conceded that older birds hydrolyse phytate to a greater extent than younger ones, thank to more phytase activity present in the digestive tract of older hens (Ravindran et al., 1995). Indeed, in laying hens fed on a maize-soyabean meal diet without a phytase supplement, the availability of phytate P was 24% in 20-week-old hens and 53% in 47-week-old hens (Marounek et al., 2008). Phytate P concentration in excreta of latter hens was significantly lower, total P and Ca concentrations, however, were not different.

The objective of the present study was to compare the total tract availability of phytate P and excreta composition in laying hens at 21 and 47 weeks of age, fed on a wheat-maize-soyabean diet without a phytase supplement. Poultry diets containing a high proportion of wheat are common in the Central Europe. Quantification of P fractions in excreta provides information regarding the solubility of P and the potential reactivity of manure once land applied (Leytem et al., 2008).

MATERIAL AND METHODS

Management and feeding

Pullets (ISA Brown) were raised on a farm purchasing one-day-old chicks from a commercial hatchery. The hatchery obtained eggs from a single commercial source, thus the genetic background of all pullets was the same. The pullets were fed a crumbled starter diet containing 20.0% crude protein (CP) until 3 weeks of age, a pullet grower pelleted diet (18.5% CP) from the 4th to the 9th week of age, a developer diet (16.0% CP, 1.0% Ca, 0.40% available P) from the 10th to the 15th week of age, and a prelay diet (16.5% CP, 2.1% Ca, 0.45% available P) in the 16th and 17 th week of age. All pullets were then fed the same wheat-maize-soyabean diet for laying hens (Table 1). The diet and fresh water

Table 1. Ingredients and determined chemical composition of hen diets

Ingredients, g/kg		Estimated composition, g/kg	
Wheat	353	Dry matter	897.1
Maize	303	Crude protein	147.1
Soyabean meal, extracted	155	Fat	57.4
Wheat bran	26	Fibre	36.9
Fish meal	15	Ash	125.7
Lucerne meal	20	Calcium	35.7
Rapeseed oil	30	Total P	4.99
Limestone	80	Phytate P	1.58
Dicalcium phosphate	10	ME, calculated, MJ/kg	11.42
Salt	2		
Vitamin-mineral mix[1]	5		
DL-methionine	1		

[1] the vitamin/mineral premix provided per kg of diet, mg: : retinyl acetate 2.5, cholecalciferol 0.06, α-tocopherol 15, niacin 20, Ca pantothenate 6, thiamine 1.5, riboflavine 4, pyridoxine 2, folic acid 0.4, biotin 0.06, cobalamin 0.01, choline-Cl 250, antioxidants 27, Mn 60, Zn 50, Fe 30, Cu 6, I 0.7, Co 0.3, Se 0.2

were available *ad libitum*. The hens were housed individually in cages, in an environmentally controlled room (20-21°C), and exposed to a daily lighting schedule of 15 h light and 9 h dark. Experimental procedures complied with the Czech code for the care and use of animals for scientific purposes.

Ten 20-week-old hens and ten 46-week-old hens were used. The diet was fed for a 7-day preliminary period followed by a 4-day test period. Clean excreta were completely collected in dishes and stored at -20°C. At the end of the test individual collections were freeze-dried and ground. The hens were weighed at the beginning of the test period. Egg production was recorded both in the preliminary and test period.

Chemical analyses

Feed and faeces were air-dried at 105°C to constant weight to estimate the dry matter content. Contents of protein, fat and fibre were determined employing instruments Kjeltec Auto 1030 Analyser, Soxtec 1043 and Fibertec 2010 from FOSS Tecator AB (Höganäs, Sweden). To determine calcium, dry excreta and feed were mineralized by HNO_3/H_2O_2, and Ca determined by the atomic absorption spectrometry using a Sollar M6 instrument (TJA Solutions, Cambridge, UK).

Total P in feeds and faeces was determined by a vanadate molybdate reagent, after ashing dry samples at 550°C (AOAC, 1980). Phytate in feeds and faeces was determined by a capillary isotachophoretic method (Dušková et al., 2001). Briefly, phytic acid was extracted from freeze-dried faeces by 0.95 M HCl, precipitated with $FeCl_3$, and ferric phytate dissolved in 1.5 M NaOH. The $Fe(OH)_3$ precipitate was removed by centrifugation, and the supernatant neutralized by adding catex Dowex 50 Wx8 in H^+-cycle. The solution was analysed by means of the column-coupling instrument EA 101 (Villa Labeco Comp., Slovakia). The phytate zone was identified on the basis of step height using a standard solution. Quantitative estimation of phytate was made *via* a regression equation between the concentration of phytate in extracts and the zone length. Phosphates present in freeze-dried faeces were extracted by 5% (w/v) trichloroacetic acid, and determined by the Fiske and SubbaRow method, as modified by Peterson (1978). To determine the phytase activity in the feed, a sample (3 g) of the feed mixture was incubated with 27 ml of physiological saline containing sodium phytate (Sigma, cat. no. P 3168) at 2 mM, in a shaking water bath at 39°C for 1 h. Thereafter, the incubation fluid was heated at 100°C for 4 min to stop enzyme reactions, and stored at -20°C before phytate determination. Samples from the beginning and the end of the incubation were analysed according to Dušková et al. (2001), except that the Fe precipitation was omitted.

Calculations and statistical analysis

Calculations of phytase activity in the diet was based on the determination of phytic acid hydrolysed to lower inositol phosphates during *in vitro* incubation of the feed. One unit of enzyme activity was defined as the amount of enzyme that hydrolysed 1 μmol phytic acid/h. The activity was related to 1 g of feed.

The *t*-test was used to evaluate statistical significancy of differences between hens old 21 and 47 weeks (GraphPad Instat, GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

The diet of hens contained total and phytate P at 4.99 and 1.58 g/kg, respectively (Table 1). Phytate P thus represented 31.7% of total P, and (according to our calculation) 68.4% of P present in phytate-containing feed ingredients.

The average body weight at the beginning of the digestibility trial was 1.56 kg in 21-week old hens, and 1.92 kg in 47-week-old hens (Table 2).

Table 2. Intake of feed, total and phytate P, phytate digestibility and excreta composition in 21 and 47 weeks old hens

Item	Age of hens, week[a]	
	21	47
Weight of hens, g[a]	1560 ± 34[b]	1916 ± 59[c]
Daily intake		
feed, g	106.6 ± 3.1[b]	141.8 ± 3.5[c]
DM, g	95.6 ± 2.8[b]	127.2 ± 3.1[c]
total P, mg	532 ± 15[b]	708 ± 17[c]
phytate P, mg	168 ± 5[b]	224 ± 6[c]
Ca, g	3.81 ± 0.09[b]	5.06 ± 0.10[c]
Excreta composition		
total P, mg/g DM	12.3 ± 0.7[b]	14.8 ± 0.6[c]
phytate P, mg/g DM	3.8 ± 0.2	3.4 ± 0.1
phytate P/total P,%	30.9 ± 3.0[b]	23.0 ± 1.5[c]
phosphate P, mg/g DM	5.9 ± 0.7[b]	8.9 ± 0.4[c]
phosphate P/total P, %	48.0 ± 3.5[b]	60.1 ± 2.2[c]
Ca, mg/g DM	78.1 ± 5.7[b]	63.0 ± 3.0[c]
Ca/P, mg/mg	6.35 ± 0.74[b]	4.26 ± 0.35[c]
Daily excreta output		
DM, g	27.1 ± 1.7[b]	34.7 ± 2.5[c]
total P, mg	333 ± 27[b]	514 ± 16[c]
phytate P, mg	103 ± 8	118 ± 5
Ca, g	2.12 ± 0.26	2.19 ± 0.13
Retention of DM, %	71.7 ± 5.2	72.7 ± 4.9
Retention of P, %	37.4 ± 4.4	27.4 ± 1.9
Retention of Ca, %	44.4 ± 5.4	56.7 ± 3.6
Phytate digestibility, %	38.7 ± 4.2	47.3 ± 3.1

means ± SEM, DM - dry matter; [a] at the beginning of the digestibility trial; [b,c] means in the same row with different superscripts differ at $P<0.05$

Feed intake per kg of body weight in these hens was similar (68.3 and 74.0 g). In the course of the experiment, hen-day egg production in younger and older layers was 0.52 and 1.00, respectively.

On average, 62.6% of total P, 61.3% of phytate P and 55.6% of Ca were recovered in faeces of 21-week-old hens. Corresponding values in 47-week-old hens were 72.6, 52.7 and 43.3%, respectively. Excreta of older hens contained significantly more total and phosphate P, and less Ca than faeces of younger hens. Concentrations of phytate P and faecal phytate output, however, were not significantly different. Phytate digestibility in 21- and 47-week-old hens was 38.7 and 47.3%, on average, respectively. The difference, however, was not statistically significant. Phytate P represented 30.9% of the total excreta P in 21-week-old hens and 23.0% of excreta P in 47-week-old hens ($P<0.05$). The main fraction of the excreta P was P of phosphates extracted by 5% TCA (48.0% and 60.1% in younger and older hens, respectively). The retention of P was almost the same in younger and older hens (199 and 194 mg/day, respectively), whereas retention of Ca was lower in younger hens (1.69±0.02 vs 2.87±0.19 Mg/day; $P<0.05$). Figure 1 shows relationship between total P and Ca in the dry matter of excreta in both groups of hens. Concentration of the total P in excreta negatively correlated with that of Ca. This correlation was marginally significant in 21-week-old hens (r - 0.58; $P=0.079$), but non-significant in 47-week-old hens

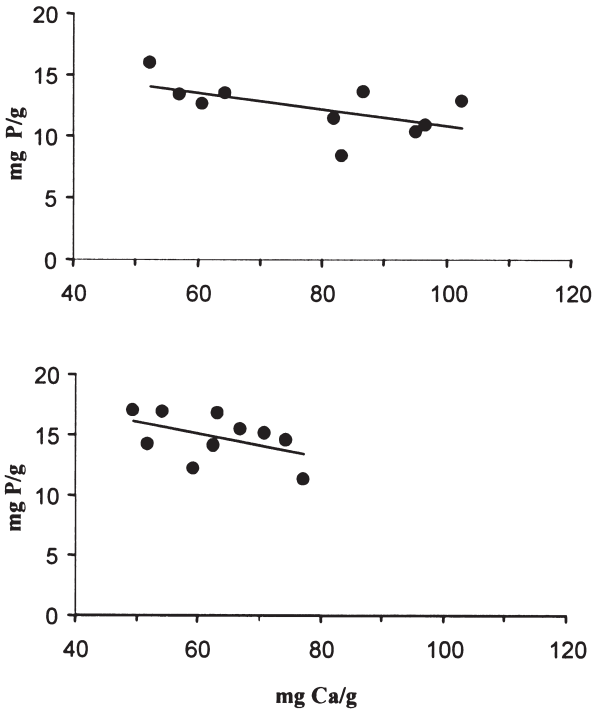

Figure 1. Relationship between total P and Ca in dry matter of excreta of 21 weeks (top) and 47 weeks (bottom) old hens

(r - 0.49; P=0.154). Phytase activity in 1 kg of the diet hydrolysed 6.12 mmol (4.04 g) phytic acid per 1 h, i.e. 102 μmol (67.3 mg) per 1 min.

DISCUSSION

A controversy exists concerning age and body weight effects on the phytate P utilization in poultry. Punna and Roland (1996) reported that heavier chickens utilized phytate P better than smaller ones. On the other hand, in 5-week-old chickens the phytate P availability negatively correlated (r - 0.52) with the body weight, supposedly due to more rapid rate of digesta passage in more rapidly growing birds (Zhang et al., 2003). In experiment of Carlos and Edwards (1998) laying hens at 24 weeks of age utilized phytate P better than hens 56 weeks old. In our experiment older hens, which were heavier, hydrolysed phytate more efficiently than younger hens. A possible reason for this might be longer retention time of digesta in the digestive tract of older hens, and the maturation of digestive functions. The present finding is consistent with our previous results (Marounek et al., 2008), however the difference in phytate P utilization between older and younger hens was less pronounced in the present study and statistically non-significant.

Age effect on excreta composition is evident both from data of Table 2 and in Figure 1. Concentration of Ca was significantly lower in 47-week-old hens than in 21-week-old hens, presumably due to higher Ca requirement in layers with greater egg production. The relatively low faecal output of total P in 21-week-old hens was probably the result of high P requirement for bone mineralization in young pullets. Consequently, proportion of dietary Ca retained was higher in older hens, and proportion of dietary P retained was higher in younger hens. The differences, however, were not quite statistically significant. Phosphates extractable by 5% TCA were the major P fraction in excreta. It has similarly been shown in pigs (Fan et al., 2001), rabbits (Marounek et al., 2003) and calves (Skrivanova et al., 2004). In experiment of Leytem et al. (2008) the majority of P in manure of broilers were organic P compounds, which included all inositol phosphates. Orthophosphates represented about 40% of total manure P. High concentration of inorganic phosphates in excreta suggests their inefficient absorption in the digestive tract, and/or the urinary excretion of excessive P. Rodehutscord et al. (2002) observed in laying hens fed diets containing P at 3.7-5.2 g/kg that absorption of P from monobasic calcium phosphate was almost complete until the terminal ileum, but P was re-directed into the excreta, likewise *via* the urine. Thus, the net absorption of P was much higher than P utilization. Since the environmental P pollution is of major concern (Nahm, 2007), this observation deserves further attention.

CONCLUSIONS

It can be concluded that the age and related different egg production significantly affect excreta composition, and amount of Ca retained. The observed effects probably reflect different P and Ca requirements of younger and older hens, and possibly also maturation of digestive functions. Further research should characterize P fractions in sections of the digestive tract of laying hens, as well as the effect of Ca on P metabolism in the intestine.

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