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Intestinal and total tract phytate digestibility and phytase activity in the digestive tract of hens fed a wheat-maize-soyabean diet*

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ABSTRACT

The intestinal and total tract digestibilities of phosphorus (P) and phytate P were determined in digestibility trials with laying hens and broiler breeders fed a diet containing P, phytate P and Ca at 6.37, 2.00 and 34.1 $g \cdot kg^{-1}$, respectively. In both digestibility trials, the total tract digestibility of phytate P was higher than the intestinal digestibility (33 and 35% vs 20 and 18%). In contrast, in both trials the total tract retention of P was lower than the intestinal retention (22 and 19% vs 52 and 42%). Phytate P represented 29.2% of the excreta P of laying hens and 23.6% of the excreta P of broiler breeders. The corresponding proportions of phosphate P were 48.7 and 46.6%, respectively. Samples of digesta and small intestinal mucosa of laying hens were diluted with physiological saline containing sodium phytate and incubated *in vitro* to determine the phytase activity. The average specific phytase activities in the crop, stomach, small intestinal contents, mucosa and caecal contents were 10.2, 9.2, 14.6, 11.5 and 135 $\mu mol \cdot h^{-1} \cdot g^{-1}$, respectively. The total phytase activities (per segment) in the small intestine (including the mucosa) and the caeca were 586 and 663 $\mu mol \cdot h^{-1}$, respectively. It can be concluded that phytase activity was present in all sections of the digestive tract. Consequently, in hens fed a wheat-maize-soyabean diet without a phytase supplement, phytate P was partially digestible. A part of the phytate degradation occurred in the hindgut.

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INTRODUCTION

Phytate phosphorus (P) is the major form of P in a range of poultry diets. In order for phytate P to be utilized, phytate has to be hydrolysed to inorganic phosphate in the digestive tract. Phytate P is relatively unavailable to poultry due to insufficient phytase activity in the digestive tract, thus, it is a common practice to supplement poultry diets with inorganic phosphates. The need for inorganic phosphate supplements can be reduced by supplementing the diet with exogenous, mostly fungal phytases (Hatten et al., 2001). Poultry are capable of utilizing part of the dietary phytate P without a phytase supplement. This has been shown both in broiler chickens (Mohammed et al., 1991; Edwards, 1993; Leske and Coon, 2002) and in laying hens (Scheideler and Sell, 1987; Leske and Coon, 1999; Marounek et al., 2008b). Although a considerable number of papers have been published in recent years dealing with supplemental exogenous phytases, only several authors attempted to elucidate the role of digestive tract phytase and identify the site of phytate P hydrolysis. Applegate et al. (2003) reported in different chicken strains an apparent ileal phytate P hydrolysis of 39.6 and 30.7%. The ileal phytate P hydrolysis was reduced to 22.0 and 24.1% when the dietary Ca concentration was increased from 4 to 9 $g \cdot kg^{-1}$, respectively. In another study, the pre-caecal disappearance of phytate P in broilers decreased linearly from 20.2 to 5.9% when the dietary Ca was increased from 4.7 to 11.6 $g \cdot kg^{-1}$ (Plumstead et al., 2008). Based on comparisons of ileal and faecal phytate hydrolysis in broiler chickens and laying hens, Kerr et al. (2000) concluded that phytate dephosphorylation continued in the hindgut. Indeed, in laying hens fed on a maize-soyabean diet the phytase activity was higher in the caeca than in other digestive segments (Marounek et al., 2008a). Leytem et al. (2008a), however, reported apparent ileal and faecal phytate P digestibility values that were not greatly different (3-30% and 10-39%, respectively) in broiler chickens fed diets without a phytase supplement. Published data on the pre-caecal and caecal phytate hydrolysis are limited. Therefore, the current experiment was conducted to compare the ileal and faecal digestibilities of phytate P and total P, and to determine phytase activity in the digestive tract of hens fed a commercial wheat-maize-soyabean diet. Poultry diets containing a high proportion of wheat are common in Central Europe. The hypothesis was tested that a significant part of phytate degradation occurred in the hindgut.

MATERIAL AND METHODS

Management and feeding

Twenty-two ISA Brown laying hens and 11 Ross 308 broiler breeders were purchased from commercial producers two weeks before the start of experiments. The hens were fed a commercial wheat-maize-soyabean diet (Table 1). The hens were housed in an environmentally controlled room (20-21°C) and exposed to a daily lighting schedule of 15 h light and 9 h dark. Feed and fresh water were available *ad libitum*. The experimental procedures complied with the Czech Code for the Care and Use of Animals for Scientific Purposes.

Table 1. Ingredients and determined chemical composition of the hen diet

Ingredients, $g \cdot kg^{-1}$		Composition, $g \cdot kg^{-1}$	
Wheat	353	Dry matter	892
Maize	303	Crude protein	167
Soyabean meal, extracted	155	Fat	52
Wheat bran	26	Fibre	31
Fish meal	15	Ash	113
Lucerne meal	20	Calcium	34.1
Rapeseed oil	30	Total P	6.37
Limestone	80	Phytate P	2.00
Dicalcium phosphate	10	ME, calculated, $MJ \cdot kg^{-1}$	11.34
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 chloride 250, antioxidants 27, Mn 60, Zn 50, Fe 30, Cu 6, I 0.7, Co 0.3, Se 0.2

Digestibility trials

Ten 37-week-old laying hens and eleven 31-week old breeder hens were housed in individual cages. Their diet was supplemented with celite at 10 $g \cdot kg^{-1}$ (an indigestible marker) and fed for a seven-day adaptation period, followed by a four-day test period. At the beginning of the test period the hens were weighed. The feed consumption was measured and the clean excreta were collected twice a day from the collection trays underneath each pen. The excreta were stored at -20°C. At the end of the trials, the daily collections were pooled in one sample per a hen, freeze-dried and ground. Following the final excreta collection, the hens were anaesthetized by inhalation of isoflurane (Nicholas Piramal India Ltd., London) and killed by cervical dislocation. The time of the killing ranged between

9 and 11 a.m. The terminal 10 cm of the ileum was removed anterior to the ileo-caecal junction. The ileal digesta was gently expressed, pooled, and frozen. The frozen samples were freeze-dried and ground.

Phytase activity measurement

Twelve 38-week-old laying hens (average weight of 1.77±0.19 kg) were anaesthetized and killed by cervical dislocation. The contents of segments of their digestive tract (crop, stomach, small intestine and caeca) and small intestinal mucosa were weighed and used for *in vitro* incubations. The small intestine was washed with tap water and the mucosa was scraped away from the underlying muscle layer with the edge of a Petri dish. The samples of the digesta and the mucosa (2 g) were diluted with 16 ml of warm (39°C) physiological saline solution, and then 3 ml of the diluted samples were taken and frozen. To increase the phytate concentration by 2 mM, 1.67 ml sodium phytate (Sigma-Aldrich) solution (20 mM) was then added to the samples. The diluted samples were incubated under a CO_2 atmosphere in a shaking water bath at 39°C for 1 h. The incubations were carried out in hermetically sealed 50 ml bottles. In the caecal incubations, the phytate concentration was doubled and the incubation time was shortened to 15 min. At the end of the incubation, 3 ml samples of the incubation fluid were removed and heated at 100°C for 4 min to stop the enzyme reactions, then stored at -20°C before the phytate determination. Due to high phosphate concentrations in digesta samples, phytase activity assay was based on measurement of phytate decomposition instead of measurement of phosphate production. This method of phytase activity measurement in digesta samples has already been used in our previous study (Marounek et al., 2008a). To determine the phytase activity in the feed, a 3 g sample was incubated with 27 ml of physiological saline containing Na-phytate at 2 mM, in a shaking water bath at 39°C for 1 h. Thereafter, the incubation fluid was heated to stop the enzyme reactions and stored at -20°C until analysed.

Analyses

The feeds and faeces were air-dried at 105°C to a constant weight to determine the dry matter content. The concentrations of protein, fat and fibre in feed were determined employing a Kjeltec Auto 1030 Analyser, a Soxtec 1043 and a Fibertec 2010 from FOSS Tecator AB (Höganäs, Sweden), respectively. To determine the calcium, feed was mineralized by HNO_3/H_2O_2 in the MDS-2000 microwave oven (LabX, Midland, ON, Canada), and the Ca was quantified by atomic absorption spectrometry (Solaar M-6, TJA Solutions, UK). The total P concentrations in the feed, ileal digesta and excreta were determined by a vanadate molybdate reagent

after ashing dry samples (AOAC, 2005; method 965.17). The phytate P in these samples was determined by a capillary isotachophoretic method (Dušková et al., 2001). The phytate in the samples from the beginning and the end of the incubations was analysed according to Dušková et al. (2001), except that the Fe precipitation was omitted. The phosphate present in the freeze-dried excreta was extracted by 5% (w/v) trichloroacetic acid, and determined according to Peterson (1978). The acid-insoluble ash determination in feed, ileal digesta, and excreta was performed after ashing the samples and treating the ash with boiling 4 M hydrochloric acid.

Calculations

The apparent ileal retention of P and digestibility of phytate P were determined by taking into account the difference between the acid-insoluble ash in the feed and ileal digesta, and the difference between the P and phytate P concentration in the feed and ileal digesta on a dry matter basis. Analogous calculations were carried out to determine the total retention of P and digestibility of phytate P. The calculations of the phytase activity in the segments of the digestive tract and in the feed were based on the estimation of phytate reduction during incubation of the digesta and mucosa samples. One unit of enzyme activity was defined as the amount of enzyme that hydrolysed 1 μmol of phytic acid per h. The specific phytase activity was related to 1 g of digesta or feed. The total phytase activity (per organ or tissue) was calculated as the product of the specific activity and the digesta or mucosa weight.

One-way ANOVA followed by Tukey's test was used to assess the significance of differences in phytase activity present in segments of the digestive tract (GraphPad Instat, GraphPad Software, Inc. La Jolla, Ca, USA).

RESULTS

The diet of the ISA Brown laying hens contained phytate and non-phytate P at 2.00 and 4.37 $g \cdot kg^{-1}$, respectively (Table 1). The phytase activity in 1 kg of the diet hydrolysed 160.2 μmol phytic acid per min to phosphate, inositol and lower inositolphosphates. Table 2 presents the intake of feed, the intake and output of total P and phytate P, and the excreta composition in digestibility trials with laying hens at the age of 38 weeks, and with broiler breeders at the age of 32 weeks (trial 1 and 2, respectively). The average feed intake of the hens used in the first and the second digestibility trial were 52.2 and 44.3 g per day per kg of body weight, respectively. The main fraction of the excreta P was P of phosphates extracted

Table 2. Intake of feed, excreta output and excreta composition in laying hens and broiler breeders[1]

Item	Digestibility trial	
	1 (layers)	2 (broiler breeders)
Weight of hens, kg	1.82 (0.22)	3.97 (0.32)
Daily intake		
feed, g	95 (29)	1.76 (7)
total P, g	0.60 (0.19)	1.12 (0.05)
phytate P, mg	189 (59)	351 (14)
Daily excreta output, g		
total P	0.47 (0.13)	0..91 (0.02)
phytate P	0.13 (0.02)	0.23 (0.03)
Excreta composition, mg·g DM^{-1}		
total P	19.5 (3.2)	17.8 (3.3)
phytate P	5.7 (0.6)	4.2 (0.4)
phosphate P	9.5 (2.1)	8.3 (3.3)

[1] data are the means and SD for 10 layers or 11 broiler breeders

by 5% TCA (48.7 and 46.6% in the first and the second digestibility trial, respectively). Phytate P represented 29.2% of the excreta P of laying hens and 23.6% of the excreta P of broiler breeders. In both digestibility trials the pre-caecal retention of P was higher than the total tract P retention (Table 3). In contrast, the total tract digestibility of phytate P was higher than the intestinal phytate P digestibility.

Table 3. Intestinal and total tract digestibility of phytate P and retention of total P in laying hens and broiler breeders

Digestibility trial	Phosphorus	Digestibility (phytate P) or retention (total P), %	
		intestinal[1]	total tract[2]
1 (layers)	phytate	20	33 (5)
	total	52	22 (5)
2 (broiler breeders)	phytate	18	35 (8)
	total	42	19 (12)

[1] pooled samples from 10 hens or 11 broiler breeders
[2] data are means and SD for 10 layers or 11 broiler breeders

The highest specific activity of phytase (per g of digesta) was in the caeca ($P<0.001$). The specific phytase activities in the crop, stomach, small intestinal contents and small intestinal mucosa were not significantly different (Table 4). A high total phytase activity (per segment) was found in the caeca, an intermediate level in the small intestinal content and mucosa, and a low level in the crop and stomach. The caeca contained almost half (45.9%) of the phytase activity of the whole digestive tract.

Table 4. Weight of the digesta and the distribution of phytase activity in the digestive tract of laying hens[1]

Segment	Digesta weight, g	Activity[2]	
		specific, per g	total, per segment
Crop	9.6 (6.0)	10.2 (1.7)[a]	98 (78)[a]
Stomach	10.5 (3.2)	9.2 (2.9)[a]	97 (44)[a]
Small intestine	24.6 (7.5)	14.6 (3.2)[a]	359 (164)[b]
Small intestinal mucosa	19.7 (4.8)	11.5 (1.5)[a]	227 (68)[ab]
Σ (pre-caecal)	64.4 (9.5)		781 (203)
Caeca	4.9 (1.7)	135.4 (15.3)[b]	663 (257)[c]
Σ (total)	69.3 (10.4)		1444 (415)

[1] data are the means and SD for 12 hens; [2] μmol·phytic acid h^{-1}
values in the same column not sharing the same superscript differ significantly at $P<0.001$

DISCUSSION

The total tract digestibility of phytate P (33 and 35% in this study) is in agreement with values reported by Scheideler and Sell (1987), Carlos and Edwards (1998) and Marounek et al. (2008b). In laying hens fed on a maize-soyabean diet, Scheideler and Sell (1987) reported a phytate P digestibility of 46.6% at 34 weeks of age, which declined at 50 and 72 weeks of age. In laying hens on a similar diet, Carlos and Edwards (1998) found that 43.0% of the phytate P was utilized. In laying hens on a wheat-maize-soyabean diet Marounek et al. (2008b) reported a phytate P digestibility of 38.7% in 21-week-old hens and 47.3% in 47-week-old hens. In laying hens on a maize-soyabean diet, the phytate P digestibility was 24% in hens 20-week-old and 53% in 47-week-old hens (Marounek et al., 2008a). The intrinsic phytase activity in wheat is high, whilst that of maize is low (Eeckhout and De Paepe, 1994). It has been shown, however, that the intrinsic phytase activity in grain does not have much influence on the phytate utilization (Leytem et al., 2008a).

The data on the apparent total P retention in hens vary in different experiments, depending on the P and Ca intake and on the phytase supplementation. In ISA Brown hens fed a maize-based diet with P and Ca at 6.4 and 40.2 $g \cdot kg^{-1}$ (without exogenous phytase), the retention of P and Ca was 43.8 and 66.7%, respectively. The corresponding P and Ca retention values in hens fed a wheat-based diet were 39.2 and 62.8% (Scott et al., 2001). In both of the digestibility trials performed in the current study, the intestinal retention of P was significantly higher than the total tract retention. Our finding is consistent with the results of the study by Rodehutscord et al. (2002) who observed that the pre-caecal absorption of P was much higher than the P utilization in laying hens. The authors concluded that the absorbed P was re-directed into the excreta, likewise *via* the urine. A similar

finding has been reported in broiler chickens by Leytem et al. (2008b).

Phosphates were the major P fraction in the excreta. It has been shown in the excreta of laying hens on a maize-soyabean diet (Marounek et al., 2008a), broiler chickens (Leytem et al., 2008b), and in other animal species (for references see Marounek et al., 2008b). As for the total P concentration in the excreta, similar values were reported in laying hens on a wheat-maize-soyabean diet by Zobač et al. (1998). The concentration of non-phytate P in the feed exceeded the level recommended by the NRC (1994), thus in the excreta total P concentration was rather high and the total tract P retention rather low (22 and 19%).

Few studies indicated the site of phytate hydrolysis in poultry. Kerr et al. (2000) pointed out the role of microorganisms in the crop and hindgut in phytate hydrolysis, and results of Leytem et al. (2008b) demonstrated that the majority of phytate P hydrolysis occurred prior to the terminal ileum, with only a 1-18% increase in the phytate P hydrolysis in the hindgut. Phytate-degrading lactic acid bacteria from the chicken intestine have been reported by Raghavendra and Halami (2009). We are not aware of a study (except our previous one - Marounek et al., 2008a) dealing with the distribution of phytase activity in the digestive tract of poultry. The results of the present study, as well as data presented previously (Marounek et al., 2008a) suggest a high caecal phytase activity, presumably of microbial origin. Indeed, in both digestibility trials reported here the total tract phytate digestibility was higher than the ileal one. However, the efficiency of post-ileal phosphate absorption is uncertain. The main sites of P absorption are at the anterior intestine (Hurwitz and Bar, 1965). Most of the pre-caecal phytate activity (75%) was present in the small intestine. The role of the crop and the stomach in phytate dephosphorylation seems to be limited.

CONCLUSIONS

Our results suggest that in laying hens and broiler breeders a part of phytate was hydrolysed post-ileally. This conclusion is based on the comparison of ileal and total tract phytate digestibility, and on measurements of the phytase activity along the digestive tract. Microorganisms responsible for caecal phytate hydrolysis should be enumerated and identified.

REFERENCES

AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis. 18th Edition. Gaithersburg, MD

Applegate T.J., Angel R., Classen H.L., 2003. Effect of dietary calcium, 25-hydroxy-cholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poultry Sci. 82, 1140-1148

Carlos A.B., Edwards Jr. H.M., 1998. The effects of 1,25-dihydrocholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. Poultry Sci. 77, 850-858

Dušková D., Marounek M., Březina P., 2001. Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. J. Sci. Food Agr. 81, 36-41

Edwards Jr. H.M., 1993. Dietary 1,25-dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilization in chickens. J. Nutr. 123, 567-577

Eeckhout W., De Paepe M., 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Tech. 47, 19-29

Hatten L.F., Ingram D.R., Pittman S.T., 2001. Effect of phytase on production parameters and nutrient availability in broilers and laying hens: a review. J. Appl. Poultry Res. 10, 274-278

Hurwitz S., Bar A., 1965. Absorption of calcium and phosphorus along the gastrointestinal tract of the laying fowl as influenced by dietary calcium and egg shell formation. J. Nutr. 86, 433-438

Kerr M.J., Classen H.L., Newkirk R.W., 2000. The effects of gastrointestinal tract micro-flora and dietary phytase on inositol hexaphosphate hydrolysis in the chicken. Poultry Sci. 79, Suppl. 1, 11 (Abstr.)

Leske K.L., Coon C.N., 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poultry Sci. 78, 1151-1157

Leske K., Coon C.N., 2002. The development of feedstuff retainable phosphorus values for broilers. Poultry Sci. 81, 1681-1693

Leytem A.B., Widyaratne G.P., Thacker P.A., 2008a. Phosphorus utilization and characterization of ileal digesta and excreta from broiler chickens fed diets varying in cereal grain, phosphorus level, and phytase addition. Poultry Sci. 87, 2466-2476

Leytem A.B., Willing B.P., Thacker P.A., 2008b. Phytate utilization and phosphorus excretion by broiler chickens fed diets containing cereal grains varying in phytate and phytase content. Anim. Feed Sci. Tech. 146, 160-168

Marounek M., Skřivan M., Dlouhá G., Břeňová N., 2008a. Availability of phytate phosphorus and endogenous phytase activity in the digestive tract of laying hens 20 and 47 weeks old. Anim. Feed Sci. Tech. 146, 353-359

Marounek M., Skřivan M., Dlouhá G., Březina P., 2008b. Digestibility of phosphorus in laying hens fed a wheat-maize-soyabean diet and the excreta phosphorus fractions. J. Anim. Feed Sci. 17, 579-587

Mohammed A., Gibney M.J., Taylor T.G., 1991. The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate-P by the chick. Brit. J. Nutr. 66, 251-259

NRC, 1994. Nutrient Requirements of Poultry. National Academy Press. Washington, DC

Peterson G.L., 1978. A simplified method for analysis of inorganic phosphate in the presence of interfering substances. Anal. Biochem. 84, 164-172

Plumstead P.W., Leytem A.B., Maguire R.O., Spears J.W., Kwanyuen P., Brake J., 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poultry Sci. 87, 449-458

Raghavendra P., Halami P.M., 2009. Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine. Int. J. Food Microbiol. 133, 129-134

Rodehutscord M., Sanver F., Timmler R., 2002. Comparative study on the effect of variable phosphorus intake at two different calcium levels on P excretion and P flow at the terminal ileum of laying hens. Arch. Anim. Nutr. 56, 189-198

Scheideler S.E., Sell J.L., 1987. Utilization of phytate phosphorus in laying hens as influenced by dietary phosphorus and calcium. Nutr. Rep. Int. 35, 1073-1081

Scott T.A., Kampen R., Silversides F.G., 2001 The effect of adding exogenous phytase to nutrient-reduced corn- and wheat-based diets on performance and egg quality of two strains of laying hens. Can. J. Anim. Sci. 81, 393-401

Zobač P., Kumprecht I., Volfová O., 1998. The effect of dietary microbial phytase on phosphorus output in laying hen droppings. Czech J. Anim. Sci. 43, 187-194