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Effects of Microbial Phytase on Apparent Digestibility of Amino Acids and Crude Protein by Female Broiler Chickens

[1]A. Hassanabadi, [2]H. Nassiri-Moghaddam, [2]H. Kermanshahi,
[2]F. Eftekhar-Shahroodi and [1]M.D. Mesgaran
[1]Department of Animal Science, Faculty of Agriculture, University of Zanjan,
P.O. Box 313, Zanjan, Iran
[2]Department of Animal Science, Faculty of Agriculture,
Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: An experiment was carried out to evaluate the effects of microbial phytase on apparent digestibility of Amino Acids (AA) and crude protein in female broiler chickens. The 240 day-old female broiler chicks were wing banded, weighted and randomly allocated to six treatment groups with 4 replicates of 10 chicks in each battery cages appropriate for completely randomized design. The treatments included supplementation of 6 dietary levels of phytase (0, 250, 500, 750, 1,000 and 1,250 FTU kg^{-1}) during 0-28 days of age. All chicks were fed a nutritionally adequate typical commercial broiler starter and grower ration (adequate in phosphorus and calcium). During days 21 to 24 excreta totally were collected for AA and CP analyzing. 250 and 500 FTU kg^{-1} phytase significantly improved digestibility of amino acids (except than alanine, valine and threonine) and CP ($p<0.05$). Phytase had no significant effect on live body weight, feed intake and feed efficiency ($p>0.05$) of the broiler chicks up to 28 days of age.

Key words: Phytase, digestibility, amino acid, crude protein, performance, broiler

INTRODUCTION

About two-thirds of the total P contained in feed ingredients of plant origin occurs as phytate (Harland and Oberleas, 1999). Poultry Feeds typically contain a high proportion of cereals, grain legumes and oilseed meals. These feed ingredients contain approximately 2.5 g kg^{-1} phytic acid (McDonald *et al*., 1990). Phytate is the term for salts of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) (Harland and Morris, 1995). The structure of phytic acid has been well described by others (Blank *et al*., 1971; Costello *et al*., 1976) and although differing conclusions have been reached regarding the alignment of the phosphate groups on the inositol nucleus, the conformation is well elucidated.

Phytic acid contains 12 dissociable protons with pKa values that range from 1.5 to around 10 (Costello *et al*., 1976). Because phytic acid is a polyanionic molecule, it can chelate di-and trivalent cations and interact with proteins, amino acids and carbohydrates, reducing the availability of these compounds for poultry (Angel *et al*., 2002; Sandberg, 2002). Furthermore, the ingestion of phytic acid by poultry can increase the excretion of endogenous compounds, further impairing the performance of the animal (Cowieson *et al*., 2004).

Phytate P is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase. In addition to low P availability, phytate limits availability of several other essential nutrients. Formation of insoluble complexes between phytate, calcium and other cations render several nutrients unavailable. Phytic acid has chelating potential and forms a wide variety of insoluble salts with di- and trivalent cations at neutral pH (Harland and Oberleas, 1999). Because corn and soybean meal make up a substantial portion of diets for chickens, the availability of P in feedstuffs of plant origin is generally very low (Harland and Oberleas, 1999; Ravindran *et al*., 1999). Bioavailability estimates of P in corn and soybean meal for pigs and poultry range from 10-30% (Jongbloed and Kemme, 1990). This low availability of phytate P poses two problems for producers: the need to add inorganic P supplements to diets and the excretion of large amounts of P in manure. In addition to low P availability, phytate limits availability of several other essential nutrients. Formation of insoluble complexes between phytate,

Correponding Author: Ahmad Hassanabadi, Department of Animal Science, Faculty of Agriculture, University of Zanjan, P.O. Box 313, Zanjan, Iran

calcium and other cations render several nutrients unavailable. Phytic acid has chelating potential and forms a wide variety of insoluble salts with di- and trivalent cations at neutral pH (Harland and Oberleas, 1999).

However, some of the detrimental effects of phytic acid can be ameliorated by the addition of exogenous phytase to the diet (Ravindran *et al*., 1999, 2001; Selle *et al*., 2000; Cowieson *et al*., 2006a). Phytase is an enzyme that hydrolyzes and releases P from the phytate molecule (Kies, 1999). Bioavailability estimates of P in corn and soybean meal for poultry range from 10 to 50% (Cowieson *et al*., 2006a). The discrepancies in the availability of phytate P reported by others are likely to be due to differences in the design of the diets offered, the concentration of endogenous phytases present in the feedstuffs offered and the age and species of livestock. Regardless of differences in reported values, the availability of P from phytate for chickens is poor because they do not possess endogenous enzymes for the effective hydrolysis of phytic acid (Bedford, 2000; Maenz, 2001).

The excretion of large amounts of P and N in effluent from intensive poultry units is indicative of the poor availability of them. This environmental problem promoted the acceptance of phytase for poultry (Selle *et al*., 2000).

The efficacy of microbial phytase to improve AA and protein digestibility has been reported by several researchers (Namkung and Leeson, 1999; Ravindran *et al*., 2001; Adeola and Sands, 2003; Cowieson *et al*., 2006a). It has been shown to be effective when the Ca and nonphytate P (nPP) concentration of the diet is reduced, thus reducing the need for inorganic P addition (Denbow *et al*., 1995; Gordon and Roland, 1998; Yan *et al*., 2001). However, the effect of phytase in nutritionally adequate Ca and nPP diets has been studied to a much lesser extent and with varied results. Some studies have shown a positive effect or an extraphosphoric effect of phytase (Waldroup *et al*., 2000; Watson *et al*., 2005), whereas other studies did not see an improvement by phytase in nutritionally adequate diets (Gordon and Roland, 1997; Sebastian *et al*., 1997).

The objective of this research was to evaluate the effects of phytase on growth performance and apparent digestibility of amino acids and CP in female broiler chickens in nutritionally normal Ca and available phosphorus diets for broiler chickens.

MATERIALS AND METHODS

Total 240 day-old female broiler chickens from Ross strain were allotted to 6 dietary treatments in completely randomized design. Each treatment was replicated 4 times with 10 chicks per replicate. Commercial brooding and management procedures were followed. Temperature was approximately 32°C on day 1 of the study and was gradually reduced to around 20°C on day 28. Lighting was set at 23 h per day. Mortality rate was recorded daily through the experiment. All chicks were fed a nutritionally adequate (NRC, 1994) typical commercial broiler starter and grower ration supplemented with different levels of phytase (0, 250, 500, 750, 1,000 and 1,250 FTU kg^{-1} of diet) (Natuphos® Phytase, 5000U g^{-1}) during 0-28 days of age. The experimental diet was prepared as a single batch in a vertical mixer. Appropriate quantities of the basal diet were selected and phytase was added prior to remixing with 0, 250, 500, 750, 1,000 and 1,250 FTU kg^{-1} of supplemental phytase. The diets were provided as a mash to 240, 1 day-old female Ross broiler chicks, which were obtained from a local hatchery, weighed and assigned to battery cages and stratified by day 1 BW such that each treatment had minimal variation in BW.

The formulation and calculated nutrient composition of the experimental diets is presented in Table 1. Chicks

Table 1: Formulations (g kg^{-1}) and calculated nutrient composition of the experimental diets

Ingredient (g kg^{-1})	Starter (0-21 day)	Grower (21-28 day)
Corn	352.3	291.1
Wheat	250.0	370.0
Soybean meal 44	231.1	226.4
Fish meal	78.1	73.7
Dicalcium phosphate	7.5	7.6
Limestone	12.1	12.4
Salt	2.0	2.0
Vitamin premix[1]	2.5	2.5
Mineral premix[2]	2.5	2.5
DL-Methionine	1.9	1.8
Sunflower oil	60.0	10.0
Determined provision*, g kg^{-1}		
Lys	14.3	14.3
Leu	19.5	19.6
Ile	10.9	11.1
Phe	11.5	11.8
Val	12.1	12.3
Tyr	6.8	6.9
Ala	12.3	12.2
Arg	13.5	13.7
Thr	9.0	9.1
His	5.9	6.0
Ser	10.3	10.6
Glu	45.0	48.0
Asp	22.2	22.1
Calculated provision, g kg^{-1}		
ME, kcal kg^{-1}	3184	2957
CP	212.3	215.8
Ca	10.0	10.0
Available P	4.6	4.5

[1]Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg, [2]Vitamins mix supplied the following per kg of diet: vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K_3, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg, *All samples analyzed in duplicate

were housed in environmentally controlled battery brooding cages (1×1 m). Chicks, feed and water were checked twice daily. Feed and water were provided on an *ad libitum* basis throughout the experiment. Body weight and feed consumption were recorded on a cage basis at weekly intervals. Corn-wheat-soybean meal diets adequate in all nutrients were used. For determination of apparent digestibility of AA and CP at 21 day of age, 4 chicks from 3 cages of each treatment were transferred to metabolic battery cages to collect the excreta. Clean stainless steel collection trays were placed under each cage (3 per treatment) and the selected birds were located individually into cages to collect the excreta. Excreta from the birds quantitatively were collected for 72 h. A subsample of excreta was collected in polyethylene bags, weighed and dried. Excreta were mixed thoroughly, frozen at -20°C and the excreta were dried to a constant weight by freeze-drier. Prior to chemical analysis, these samples were ground (0.5 mm, screen). Feed consumption was determined and digestibility coefficients of nutrients were calculated. Amino acid concentrations in the excreta and the diet were determined by HPLC (Roth, 1971; Jones *et al*., 1981) following acid hydrolysis and precolumn derivatization using orthophthaldialdehyde. The HPLC system comprised a Varian 5000 high performance liquid chromatography and a Varian Fluori-chrom detector (excitation 340 nm emission 450 nm). The flow rate of the pump was 1.5 mL min^{-1} and the column used was a Supelcosil 3 μ LC-18 reverse phase column (4.6×150 mm; Supelco) equipped with a guard column (4.6×50 mm). Because this method of hydrolysis destroys methionine and cystine, data on these amino acids are not reported. The concentration of N in the excreta and the diet samples were determined using Kjeltec Auto Analyzer 1030/ Dijestion System 20 (AOAC, 2003).

Statistical analysis: Data were analyzed as completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The cage of chicks served as the experimental unit. Means were compared using Duncan's new multiple range test (Steel and Torrie, 1980). The level of significance was reported at $p<0.05$.

RESULTS AND DISCUSSION

The effects of supplemental phytase on performance characteristics of the chicks up to 28 days of age are presented in Table 2. The addition of phytase to the experimental diets had no significant effect on live body weight, daily weight gain, feed intake and feed conversion ratio up to 28 days of age ($p>0.05$).

As seen in other studies, deficiencies of Ca and nPP decreased ADG, ADFI and G:F in chicks (Johnston and Southern, 2000). Phytase addition did not increase ADG and ADFI in this study and it was probably due to this fact that the present experiment was continued only up to 28 days of age. However, other studies have reported that the response to phytase on ADG and ADFI was more pronounced in the deficient Ca and nPP diets (Gordon and Roland, 1997; Sebastian *et al*., 1997). While, at the present study the experimental diets nutritionally were adequate in the case of all nutrients as well as Ca and nPP.

Table 2: Effect of phytase dose on BW, BW gain, feed intake and Feed Conversion Ratio (FCR) of growing broiler chickens from d 1-28 fed corn-wheat-soybean meal based diets

Treatment (FTU phytase)	BW (g day 28)	BW gain, g/bird/d day 0-28	Intake, g/bird/d day 0-28	FCR day 0-28
0 (Control)	854.97	29.01	53.77	1.85
250	855.44	28.97	52.80	1.82
500	857.83	29.12	52.79	1.82
750	872.03	29.65	54.10	1.82
1,000	907.17	30.89	55.93	1.81
1,250	863.08	29.28	53.97	1.84
SE	20.16	0.72	2.03	0.07

Means within each column with no superscript are not significantly different ($p>0.05$), [SE]Standard Error

Table 3: Effect of phytase dose on the amino acid and CP apparent digestibility (%) by growing broiler chickens

Treatment (FTU Phytase)	Lys	Leu	Ile	Phe	Val	Tyr	Ala	Arg	Thr	His	Ser	Glu	Asp	Total	CP
0 (Control)	88.1^{b}	86.4^{b}	84.3^{b}	87.2^{b}	79.8	80.9^{b}	86.2	91.4^{c}	86.0	88.0^{d}	85.4^{b}	87.9^{c}	83.5^{b}	85.8^{b}	68.1^{b}
250	92.2^{a}	90.4^{a}	89.0^{a}	91.4^{a}	84.1	87.7^{a}	85.7	95.1^{ab}	88.0	92.4^{ab}	89.6^{ab}	92.1^{ab}	88.3^{ab}	89.7^{a}	72.2^{a}
500	93.1^{a}	90.8^{a}	89.5^{a}	91.8^{a}	84.3	88.4^{a}	85.7	96.0^{a}	88.6	93.2^{a}	90.4^{a}	92.7^{a}	88.7^{a}	90.2^{a}	73.1^{a}
750	90.1^{ab}	88.1^{ab}	86.0^{ab}	89.1^{ab}	83.3	83.3^{ab}	82.9	92.4^{bc}	84.2	89.7^{dc}	85.8^{b}	89.1^{bc}	84.5^{ab}	86.8^{ab}	70.1^{ab}
1,000	91.4^{ab}	88.5^{ab}	86.1^{ab}	90.3^{ab}	83.0	83.6^{ab}	82.1	94.5^{ab}	86.4	90.8^{abc}	87.8^{ab}	90.5^{abc}	86.4^{ab}	87.8^{ab}	71.3^{ab}
1,250	90.7^{ab}	88.9^{ab}	87.1^{ab}	90.4^{ab}	83.9	83.1^{ab}	88.5	93.7^{abc}	86.9	90.0^{bcd}	88.0^{ab}	90.5^{abc}	86.7^{ab}	87.0^{ab}	70.7^{ab}
SE	0.99	0.96	1.10	1.03	1.98	1.77	3.24	0.83	1.73	0.81	1.29	0.96	1.43	1.08	1.01
% of digestibility increasing at 250 FTU	4.6	4.6	5.6	4.8	-	8.4	-	4.0	-	5.0	4.9	4.8	5.7	4.5	6.0

[a-c]Means within each column with no common superscript differ significantly ($p<0.05$), [SE] Standard Error

The effect of phytase on amino acid digestibility is presented in Table 3. The coefficients of digestibility of all amino acids (except than alanine, valine and threonine) were improved by the addition of phytase and in most instances, there was little to be gained from adding phytase above 250 FTU kg^{-1}, with significant beneficial effects often attained at only 250 or 500 FTU kg^{-1}. The digestibility coefficients of amino acids were not all affected to the same degree by the addition of phytase and improvements ranged from 0% for alanine, valine and threonine to almost 8.4% for tyrosine (Table 3). The average improvement in coefficients of digestibility of amino acids by 250 FTU was approximately 4.5% compared with the control. From the data presented in this paper, it appears that low doses of the phytase studied (250 to 500 FTU kg^{-1}) are sufficient to improve amino acid and CP digestibility coefficients (4.5 and 6%, respectively), with higher doses (750 to 1,250 FTU kg^{-1}) do not have additional effect.

Recent work has shown that the inclusion of phytase, with xylanase, amylase and protease, to nutritionally deficient and nutritionally rich diets can improve performance and uniformity of BW. The improvements may be due to an improvement in the net energy value of the diet (Cowieson and Adeola, 2005; Cowieson *et al*., 2006 b,c). It may be that to realize the full potential of phytase on P retention, diets should be designed to be adequately nutrient dense and balanced with regard to their supply of amino acids and energy, in the presence of phytase. Furthermore, it is possible that to maximize the response to phytase it should be added to the diet in combination with accessory enzymes such as xylanase or other enzymes that are capable of improving access to dietary phytate.

It is interesting that although the digestibility coefficients of most of amino acids were improved by the addition of phytase, there was a large variation in response that was dependent on the amino acid. The digestibility coefficient of arginine was improved by only 4% by the addition of phytase, whereas for tyrosine, the improvement was 8.4%. This is in agreement with findings by Ravindran *et al.* (1999) and Namkung and Leeson (1999), who found that the digestibility improvements of the amino acids have variations. Although published results on the effect of phytase on amino acid digestibilities vary (Selle *et al*., 2000; Adeola and Sands, 2003), it is clear that, when phytase influences amino acid digestibility coefficients, it does not do so to the same extent for all amino acids. This may be linked to differential interactions between amino groups and phytate or it may be associated with the ability of phytate to increase the loss of endogenous compounds, such as mucins, that are rich in certain amino acids (Mansoori and Acamovic, 1998; Cowieson *et al*., 2004). Consistent with the effect of phytase on amino acid digestibility, the effect on CP digestibility can be variable.

The consequence of this is that most of the improvements in amino acid and energy retention associated with phytase addition may be expected to be achieved by the removal of 1 or 2 phosphate groups, with further improvements increasingly unlikely as each subsequent phosphate is cleaved. This means that the addition of relatively low doses of phytase has the capacity to improve the retention of nonmineral nutrients and that the addition of higher doses may only have a significant effect on P retention and not on energy or amino acids. However, these effects are likely to be linked to the phytate concentration in the diet and also to the Ca:P ratio and the ratios among available nutrients. Diets containing rice bran or canola meal may benefit more from higher doses of phytase than for those based on corn, wheat and soybean meal, because rice bran and canola contain higher concentrations of phytate P (Eeckhout and De Paepe, 1994).

CONCLUSION

It can be concluded that supplemental phytase is effective in improving the amino acid CP digestibility coefficients. The benefits on amino acids and CP digestibility coefficients are maximized at relatively low doses.

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