

## Supplementation of Corn-Wheat-Soy-Based Diets with Phytase: Effects on Digestibility of Amino Acids in Broiler Chicks

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**Abstract:** An experiment was conducted to evaluate the effects of microbial phytase on productive traits and apparent digestibility of Amino Acids (AA) in male broiler chickens. The experiment was a completely randomized design. Each treatment consisted of 5 replicates of 12 male broilers, for a total of 300 Ross broiler chicks. Broiler chickens were fed similar diets containing 5 levels of phytase (0, 250, 500, 750 and 1,000 FTU/kg of diet) from 1-28 days of age. Excreta were quantitatively collected from 21-24 days of the experiment. The excreta stored at -20°C, freeze-dried and analyzed for amino acids. During the experiment, Body Weight (BW), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) were measured weekly. Total 250 and 500 FTU phytase supplementation significantly increased ( $p < 0.05$ ) apparent AA digestibility. Higher levels of phytase decreased AA digestibility in compare to 250 and 500 FTU. Phytase had no significant effect ( $p > 0.05$ ) on body weight, feed intake and feed efficiency up to 28 days of age.

**Key words:** Broiler, phytase, amino acid, poultry feeds, phosphate

### INTRODUCTION

Poultry Feeds typically contain a high proportion of cereals, grain legumes and oilseed meals. These feed ingredients contain approximately  $2.5 \text{ g kg}^{-1}$  phytic acid (McDonald *et al.*, 1990). About two-thirds of the total P contained in feed ingredients of plant origin occurs as phytate (Harland and Oberleas, 1999). 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).

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 10-50% (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 in male broiler chickens in nutritionally normal Ca and available phosphorus diets for broiler chickens.

## MATERIALS AND METHODS

The study was conducted with the approval of the Animal Ethics Committee of the Ferdowsi University of Mashhad, Iran. Three hundred male broiler chickens from Ross strain were allotted to 5 dietary treatments in completely randomized design. Each treatment was replicated 5 times with 12 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 FTU/kg of diet) for 28 days. 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 and 1,000 FTU/kg of supplemental phytase. The diets were provided as a mash to 300, 1-day-old male Ross broiler chicks, which were obtained from a local hatchery, weighed and assigned to floor pens and stratified by day 1 BW such that each treatment had minimal variation in BW.

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

Table 1: Formulations (g kg<sup>-1</sup>) and calculated nutrient composition of the experimental diets

Ingredient (g kg <sup>-1</sup> )	Starter (day 0-21)	Grower (day 21-28)
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 <sup>1</sup>	2.5	2.5
Mineral premix <sup>2</sup>	2.5	2.5
DL-Methionine	1.9	1.8
Sunflower oil	60.0	10.0
<b>Determined provision* (g kg<sup>-1</sup>)</b>		
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
<b>Calculated provision (g kg<sup>-1</sup>)</b>		
ME, kcal kg <sup>-1</sup>	3184	2957
CP	212.3	215.8
Ca	10.0	10.0
Available P	4.6	4.5

<sup>1</sup>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; <sup>2</sup>Vitamins mix supplied the following per kg of diet: vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K<sub>3</sub>, 4 mg; vitamin B<sub>12</sub>, 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 floor pens (1.5×1.5 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 pen basis at weekly intervals. Corn-wheat-soybean meal diets adequate in all nutrients were used. For determination of apparent digestibility of amino acids, 3 chicks from 3 floor pens of each treatment were transferred to battery cages at 21 day of age 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 totally 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 Fluorichrom detector (excitation 340 nm emission 450 nm). The flow rate of the pump was 1.5 mL min<sup>-1</sup> and the column used was a Supelcosil 3 micron 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 was determined using Kjeltac Auto Analyzer 1030/ Digestion 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 pen 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 phytase supplementation on growth performance of the chickens are summarized in Table 2. The addition of 750 FTU kg<sup>-1</sup> of supplemental phytase to the experimental diets improved daily weight gain up to 28 days of age (p<0.05) in compare to 1,000 FTU/kg and the increase in ADG accompanied with no effect on feed intake and feed efficiency. This result is in agreement with Denbow *et al.* (1995) and Sebastian *et al.* (1996, 1997). However, the difference was not significantly different with control or other levels of the supplemental phytase. The addition of phytase to the experimental diets had no significant effect (p>0.05) on feed intake, feed conversion ratio and live body weight in whole experiment period. 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).

The effect of phytase on amino acid digestibility coefficients is presented in Table 3. The coefficients of digestibility of all amino acids were improved by the addition of phytase and in most instances, there was little to be gained from adding phytase above 250 FTU kg<sup>-1</sup>, with significant beneficial effects often attained at only 250 or 500 FTU/kg. The digestibility coefficients of amino acids were not all affected to the same degree by the addition of phytase and improvements ranged from 3.4% for arginine to almost 10.4% for valine (Table 3). The average improvement in coefficients of digestibility of amino acids was approximately 5.6% compared with the control. From the data presented in this paper, it appears that low doses of the phytase studied (250-500 FTU/kg) are sufficient to improve amino acid and CP digestibility coefficients (5.6 and 10.7%, respectively), with higher doses (750-1,000 FTU/kg) 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

Table 2: Effect of phytase dose on BW, BW gain, feed intake and feed conversion ratio (FCR) of growing broiler chickens from d 1 to 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)	958.46	39.10 <sup>ab</sup>	58.14	1.71
250	957.67	38.90 <sup>ab</sup>	57.54	1.72
500	937.43	39.40 <sup>ab</sup>	58.14	1.82
750	953.36	40.60 <sup>a</sup>	57.94	1.78
1,000	924.59	36.30 <sup>b</sup>	56.99	1.80
SE	19.65	1.00	1.97	0.06

<sup>a,b</sup> Means within each column with no common superscript differ significantly (p<0.05); <sup>SE</sup> Standard Error; <sup>1</sup>One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1 μmole of inorganic P per minute from sodium phytate at pH 5.5 and 37.°C

Table 3: Effect of phytase dose on the apparent coefficient of amino acid and CP 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)	87.8 <sup>b</sup>	85.7 <sup>b</sup>	84.0 <sup>ab</sup>	86.4 <sup>b</sup>	77.4 <sup>b</sup>	81.2 <sup>b</sup>	92.2 <sup>a</sup>	90.6 <sup>c</sup>	82.2 <sup>b</sup>	88.0 <sup>c</sup>	84.1 <sup>b</sup>	87.2 <sup>bc</sup>	82.1 <sup>c</sup>	84.3 <sup>b</sup>	68.3 <sup>b</sup>
250	90.7 <sup>a</sup>	89.4 <sup>a</sup>	87.2 <sup>a</sup>	90.0 <sup>a</sup>	85.4 <sup>a</sup>	86.8 <sup>a</sup>	84.4 <sup>ab</sup>	93.7 <sup>ab</sup>	86.3 <sup>ab</sup>	91.0 <sup>a</sup>	90.0 <sup>ab</sup>	90.0 <sup>ab</sup>	86.2 <sup>ab</sup>	88.6 <sup>a</sup>	75.4 <sup>a</sup>
500	91.3 <sup>a</sup>	89.3 <sup>a</sup>	87.0 <sup>a</sup>	91.0 <sup>a</sup>	85.5 <sup>a</sup>	85.6 <sup>a</sup>	83.9 <sup>ab</sup>	94.4 <sup>a</sup>	87.4 <sup>a</sup>	91.4 <sup>a</sup>	90.7 <sup>a</sup>	90.7 <sup>a</sup>	87.1 <sup>a</sup>	89.0 <sup>a</sup>	75.6 <sup>a</sup>
750	87.9 <sup>b</sup>	85.7 <sup>b</sup>	83.4 <sup>b</sup>	86.4 <sup>b</sup>	80.5 <sup>ab</sup>	84.0 <sup>ab</sup>	79.2 <sup>b</sup>	90.8 <sup>bc</sup>	82.3 <sup>b</sup>	88.7 <sup>bc</sup>	86.6 <sup>c</sup>	86.6 <sup>c</sup>	82.4 <sup>bc</sup>	85.0 <sup>b</sup>	70.5 <sup>ab</sup>
1,000	89.7 <sup>b</sup>	87.4 <sup>ab</sup>	85.0 <sup>ab</sup>	88.6 <sup>ab</sup>	82.8 <sup>ab</sup>	86.1 <sup>a</sup>	82.3 <sup>abc</sup>	93.2 <sup>b</sup>	85.3 <sup>ab</sup>	90.3 <sup>abc</sup>	89.2 <sup>abc</sup>	89.2 <sup>abc</sup>	85.0 <sup>abc</sup>	87.4 <sup>ab</sup>	72.8 <sup>ab</sup>
SE	0.75	0.94	1.04	0.92	1.94	1.08	2.88	0.93	1.24	0.64	1.04	0.87	1.20	0.94	1.90
% of digestibility increasing	4.0	4.3	3.8	5.3	10.4	6.9	-8.4	3.4	6.3	3.8	7.8	4.0	6.1	5.6	10.7

<sup>a,c</sup> Means within each column with no common superscript differ significantly (p<0.05); <sup>SE</sup> Standard Error

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 coefficients of digestibility of all 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 3.4% by the addition of phytase, whereas for aspartic acid, threonine, tyrosine, serine and valine, the improvements were between 6.1 and 10.4%. This is in agreement with findings by Ravindran *et al.* (1999) and Namkung and Leeson (1999), who found that the digestibility of these amino acids in particular was improved by phytase. Furthermore, Rutherford *et al.* (2002) found that phytase improved the digestibility coefficients of aspartic acid, valine and threonine compared with unsupplemented corn. 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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