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Efficacy of Fungal Phytase on Apparent Ileal Digestibility of Nutrients in Broiler Chicken

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ABSTRACT

This experiment was conducted to assess efficacy of phytase of fungal (*Aspergillus foetidis*)-origin on apparent ileal digestibility of nutrients in broiler chicken fed with a maize-soybean based diet. A feeding trial was conducted on 126 chicks, divided into three dietary treatments with six replicates having seven chicks in each following a completely randomized design. Semi purified phytase of *Aspergillus foetidis* (MTCC 11682) was used for feeding. The dietary treatments consisted of one normal phosphorus (NP) group without any phytase enzyme, two low phosphorus (LP) groups LP500 and LP1000 with supplemental laboratory phytase at 500 and 1000 FTU/kg, respectively. The apparent ileal digestibility coefficient (AIDC) of most amino acids were improved significantly ($P<0.05$) in phytase supplemented group except crude protein, methionine, glutamic acid, arginine, isoleucine. The AIDC of calcium, phosphorus and iron were improved significantly ($P<0.05$) due to phytase supplementation. The P excretion g/kg FI was higher ($P<0.001$) in NP fed followed by LP 1000 and least excretion was noticed in LP 500 fed group. It may be concluded that immobilized partial purified fungal phytase at 1000 FTU/kg in low phosphorus diet was effective in replacing 0.12% non-phytate phosphorus in the diet of broiler chicken for better ileal digestibility of amino acids and minerals with reduced P excretion.

Key words: Amino acids, Broiler, Ileal digestibility, Minerals, Phytase

INTRODUCTION

Phytate phosphorus (PP) is the primary storage form of phosphorus in cereal grains and legumes. Phytate (myo-inositol hexaphosphate; IP-6) impairs the digestion of several minerals, amino acids and also reduces activity of pepsin, trypsin and α-amylase (Luo *et al.,* 2007). The PP is not absorbed in the gut of poultry which is excreted, leading to environmental pollution. The inorganic sources of P, which are expensive, are added to feeds to meet P requirements (Ali *et al.,* 2017). The best way to mitigate the negative effects of PA is supplementation of phytase enzyme in the poultry diet (Attia *et al.,* 2020). Selle and Ravindran (2007) extensively reviewed the efficacy of phytase on bioavailability of P and Ca in poultry, but its ability to enhance amino acid digestibilities and energy utilisation have not been completely understood (Liu *et al.,* 2015). There had been very few works reported on the laboratory-scale production of enzyme and subsequent testing in broiler chicken (Ahmad *et al.,* 2000; Lan *et al.,* 2012; Lalpanmawia *et al.,* 2014). The objective of present study was to test the efficacy of phytase produced from *Aspergillus foetidis (*MTCC 11682) on ileal digestibility of nutrients in broiler chicken.

MATERIALS AND METHODS

Phytase enzyme was produced by employing fungal (*Aspergillus foetidis* MTCC 11682) immobilization technique standardized earlier by Lalpanmawia *et al.* (2014). Purification of phytase enzyme was done by using 85% ammonium sulphate as a precipitating medium (Soni *et al.,* 2010). Enzyme activity of semi purified phytase was estimated before incorporation into the feed (Kim and Lei, 2005).

The animal care and use protocol (No. 5/2012) was reviewed and approved by the Animal Ethics committee of National Institute of Animal Nutrition and Physiology, Bangalore, India. In total 126, unsexed, day old Cobb broiler chicks were distributed into three treatments in random manner; each treatment had six replicates with seven chicks in each replicate. The birds

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were housed in battery cages with *ad libitum* access to feed and water. The birds were reared with 24h light and proper air ventilation under standard management conditions.

The dietary treatments consisted of one normal phosphorus diet (NP), without any phytase enzyme (0.45% available P during starter and 0.40% during finisher phase), and two low phosphorus diets (0.32% available P during starter and 0.28% during finisher phase) supplemented with laboratory phytase at 500 FTU (LP500) and 1000 FTU (LP1000), respectively. Ingredient and nutrient composition of experimental diets is given in Table 1. The phytate phosphorus content of maize and soybean meal was estimated by the method described by Haug and Lantzsch (1983). The Ca and P in feed were analysed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer Instrument. The amino acid content of feed and digesta was analyzed at Amino Lab, Evonik Industries. The results of growth trial has already been published (Manobhavan *et al.,* 2015).

At the end of 35 days, four birds were selected randomly per pen and sacrificed by cervical dislocation for evaluation of ileal digestibility of nutrients. After sacrificing the birds, the contents of the ileum from Meckel's diverticulum to ileo-caecal junction were pooled within a pen, collected and frozen immediately (-20°C). The digesta samples were freeze-dried, ground and stored in an air tight container for estimation of crude protein (AOAC, 1990), amino acids (Amino Lab, Evonik

Table 1. Ingredient and nutrient composition of experimental diets during starter (0-3 wk) and finisher (4-5 wk) age broilers

Ingredient composition(g/kg)	Starter (0-3 wk)		Finisher (4-5 wk)	
	NP	LP	NP	LP
Maize	583.4	584.2	612	612.5
Soybean meal	359.3	360	325	325
Sunflower oil	20	20	25	25
Limestone	10	16	10	17
Di calcium phosphate	17.5	10	15	7.5
Celite	0	0	5	5
Salt	3.5	3.5	3.5	3.5
Lysine	2.5	2.5	1	1
Methionine	1.3	1.3	1	1
Vitamin mineral premix[a]	2.5	2.5	2.5	2.5
Nutrient composition (g/kg)				
ME (MJ/ kg)	12.6	12.6	12.8	12.8
CP	222	223	193	199
Lysine	13.7	13.7	11.3	11.3
Methionine	4.9	4.9	4.3	4.3
Calcium	10.9	10.7	10.1	10.3
Total phosphorus	7.57	6.30	6.96	5.68
Phytate phosphorus	3.12	3.12	2.98	2.98
Available phosphorus	4.45	3.18	3.97	2.69

[a]Trace mineral premix, 1 g/kg; Vitamin premix1g/kg and choline 0.5g/kg. Trace mineral premix supplied mg/ kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. the vitamin premix supplied per kg diet: vitamin A, 8250 IU; vitamin D3, 1200 ICU; vitamin K, 1mg; vitamin E, 40 IU; vitamin B1, 2 mg; vitamin B2 4 mg; vitamin B12, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg; NP – Normal phosphorus fed group; LP – Low phosphorus fed group

Industries), acid insoluble ash (AIA) (AOAC, 1990) and minerals (ICP). The P measured in the ileal digesta was used for calculating the total P in the excreta/faeces. The apparent ileal digestibility coefficient (AIDC) of the nutrients was calculated from the equation:

$$AIDC = 1 - [(ileal\ nutrient/\ ileal\ AIA) / (diet\ nutrient/\ diet\ AIA)]$$

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) for completely randomized design and tested for significance between the dietary treatments means employing Tukey's HSD Post-hoc test (SPSS, 2010 Version 18.0).

RESULTS AND DISCUSSION

The apparent ileal digestibility coefficient of most amino acids differed significantly (P<0.05) among the groups except methionine, glutamic acid, arginine, isoleucine and CP (Table 2). The AIDC of amino acids in broilers fed LP 1000 was statistically similar (P<0.05) to NP fed group. Other research workers (Woyengo *et al.,* 2010; Rutherfurd *et al.,*2012; Lalpanmawia *et al.,* 2014) have reported that there was no significant effect of phytase on ileal CP digestibility. In contradictory to our findings, Ravindran *et al.* (2006) reported improved ileal CP digestibility in broiler. They reported that phytase supplementation at 500FTU/kg was effective when the diet contained medium to high concentrations of phytate (2.8, 3.3, and 3.8 g of phytate P/kg). Cowieson *et al.* (2006) found that digestibility coefficients of amino acids were improved by phytase supplementation in low phosphorus diet. In addition, they reported that digestibility coefficient of arginine was improved by only 2% by the addition of phytase, whereas for valine, threonine, isoleucine, aspartic acid, and alanine the improvements were between 4.5 and 5.6%. Namkung and Leeson (1999) found that the digestibility of valine and isoleucine in particular was improved by phytase (1149 FTU/kg). Rutherfurd *et al.* (2002) found that phytase supplementation (750 FTU/kg) improved the digestibility coefficients of aspartic acid, valine and

Table 2. Effect of fungal phytase on apparent ileal digestibility coefficient (AIDC) of crude protein and amino acids in broilers at 5 weeks of age

Groups	NP	LP 500	LP 1000	SEM	P value
Crude protein	0.824	0.801	0.819	0.0053	0.1977
Cystine	0.804^{a}	0.733^{b}	0.755^{ab}	0.0124	0.0364
Serine	0.867^{a}	0.821^{b}	0.848^{ab}	0.0078	0.0245
Proline	0.911^{a}	0.877^{b}	0.897^{ab}	0.0054	0.0088
Alanine	0.853^{a}	0.799^{b}	0.826^{ab}	0.0093	0.0235
Aspartic acid	0.880^{a}	0.842^{b}	0.865^{ab}	0.0067	0.0435
Glutamic acid	0.913	0.878	0.894	0.0061	0.0320
Methionine	0.914	0.889	0.907	0.0051	0.1154
Lysine	0.913^{a}	0.888^{b}	0.901^{ab}	0.0043	0.0310
Threonine	0.814^{a}	0.768^{b}	0.802^{ab}	0.0086	0.0435
Arginine	0.902	0.883	0.886	0.0043	0.1436
Isoleucine	0.876	0.836	0.861	0.0075	0.0589
Leucine	0.893^{a}	0.849^{b}	0.872^{ab}	0.0076	0.0309
Valine	0.857^{a}	0.815^{b}	0.842^{ab}	0.0075	0.0372
Histidine	0.886^{a}	0.838^{b}	0.856^{ab}	0.0085	0.0340
Phenylalanine	0.845^{a}	0.801^{b}	0.831^{ab}	0.0077	0.0307
Glycine	0.819^{a}	0.775^{b}	0.807^{ab}	0.0080	0.0307

^{a,b}Means in the same row bearing different superscript differ significantly

Table 3. Effect of fungal phytase on apparent ileal digestibility coefficient (AIDC) of minerals in broilers at 5 weeks of age

Groups	NP	LP 500	LP 1000	SEM	P value
Ca	0.574[a]	0.526[b]	0.579[a]	0.0078	0.0141
P	0.464[b]	0.515[a]	0.511[a]	0.0075	0.0020
Mg	0.272	0.232	0.333	0.0214	0.1519
Mn	0.471	0.499	0.538	0.0261	0.3754
Zn	0.455	0.378	0.478	0.0246	0.2260
Fe	0.241[ab]	0.258[a]	0.199[b]	0.0093	0.0183

[a,b]Means in the same row bearing different superscript differ significantly; SEM: standard error of means;

threonine. The effect of phytase on amino acids digestibility varies (Selle *et al.,* 2000; Adeola and Sands, 2003); it is clear that phytase influences amino acids digestibility coefficients and moreover it is not the same 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 (Cowieson *et al.,* 2004). Cowieson *et al.* (2017) reported that the digestibility of all amino acids was increased by the addition of phytase. In addition, they found that increasing the inclusion level of phytase from 500 to 1000 FTU/kg resulted in an increase in the amino acid response from around 3% to around 5%. Current study revealed that in low P diet phytase supplementation @ 500 FTU and 1000 FTU improved the AIDC of amino acids except methionine, glutamic acid, arginine and isoleucine. In contrary, Lalpanmawia *et al.* (2014) observed that apparent ileal digestibility co-efficient (AIDC) of amino acids was not significantly affected by phytase supplementation in low P diet (1.2 g/kg non-phytin P less). Sebastian *et al.* (1997) reported that microbial phytase (at 600 FTU/kg diet) supplementation had no effect ($P<0.065$) on the apparent ileal digestibility (AID) of CP or any AA except Met and Phe in male broiler chickens, whereas, in females, adding phytase increased the AID of all AA except Lys, Met, Phe and Pro.

The apparent ileal digestibility coefficient of calcium, phosphorus and iron differed significantly ($P<0.05$) (Table 3). The AIDC of P was higher in LP 1000 and LP 500 than NP fed groups. The AIDC of magnesium, manganese and zinc were not significantly ($P>0.05$) different among the groups. The studies of Ravindran *et al.* (2006) and Lalpanmawia *et al.* (2014) found improved P and Ca digestibility in phytase supplemented diet. On the contrary, Onyango *et al.* (2005) observed no improvement in AID of Ca and P by phytase supplementation in diet. Tiwari *et al.* (2010) and Woyengo *et al.* (2010) reported improved AID of P by phytase supplementation, but AID of Ca was not affected by phytase supplementation.

The total P intake was higher ($P<0.001$) in NP fed group followed by LP 1000 and lower in LP 500. The total P excretion also followed the same trend. The P excretion (g/kg feed intake) was higher ($P<0.001$) in NP fed group followed by LP 1000 group and least excretion was noticed in LP 500 fed group. Many researchers (Kwon *et al.,* 1995; Gunashree *et al.,* 2007) reported that a reduction of P excretion by phytase

Table 4. Effect of fungal phytase on total P intake and excretion in broilers

Groups	NP	LP 500	LP 1000	SEM	P value
Intake, g/bird	19.4[a]	14.40[c]	15.94[b]	0.5187	0.001
Excretion, g/bird	10.4[a]	6.99[c]	7.79[b]	0.3573	0.001
Excretion, g/kg FI	3.85[a]	2.87[c]	2.89[b]	0.1106	0.001

[a,b,c]Means in the same row bearing different superscript differ significantly; SEM: standard error of means; FI, feed intake

supplementation. The reduction in P losses in the excreta can reduce the environmental pollution caused by P. Ibrahim *et al.* (1999) reported 56% reduction in total P excretion on 600 FTU/kg phytase supplementation in low P diet. Several other researchers (Pintar *et al.,* 2005; Sukumar *et al.,* 2013) reported reduction in total P excretion in low P diet compared to control and the reduction was still higher on phytase supplementation in the low P diet.

CONCLUSION

It may be concluded that supplementation of immobilized partial purified fungal phytase at 1000 FTU/kg in low phosphorus diet was effective in replacing 0.12% non-phytate phosphorus in the diet of broiler chicken for better ileal digestibility of amino acids and minerals with reduced P excretion.

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