



# Effect of Phytase Supplementation on Growth Performance in Broiler Chickens

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## ABSTRACT

Day-old broiler birds (n = 96) were randomly divided into four groups, each group with four replicates and having 6 birds in each replicate. The birds were supplemented with corn-based diet. On day 35, birds were slaughtered to determine the relative weights of heart, liver, kidneys, spleen, gizzard and intestines. Results showed that supplementation of phytase enzymes affected the weight and length of small intestine and feed conversion ratio (FCR) and did not affect the other zoo technical variables like body weight, feed consumption, and weight gain during the whole experimental period. Application of phytase failed to exert any influence on the weights of gizzard, proventriculus, heart, liver, spleen and empty intestine. The weights and lengths of the small intestine were highly significant ( $P > 0.05$ ) in the treatment groups. This study showed the potency of phytase to enhance the growth performance in broilers and it will lay foundation for future research on poultry feed in Pakistan.

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## Authors' Contribution

HZ designed the study. KK performed the study. ZR and HM wrote the paper. HR edited the manuscript.

## Key words

Phytase, Broiler, Performance.

## INTRODUCTION

Poultry feed contains plant ingredients as their major component, such as corn and soya bean meal in which there is about 67% of the total phosphorus which is present chelated form with phytate (Ravindran *et al.*, 1999). Phytic acid is present in plant ingredients such as seeds and grains as a chelated salt which is called as phytate, which is also called as the phytic acid molecule get chelated to protein, lipid, carbohydrates or both lipid and carbohydrates and mineral cations (Selle *et al.*, 2000). In animal feeds of plant origin the main source of phosphorus is phytate, but this phosphorus is not available for absorption in the intestine unless it hydrolyzes and the phosphate group is removed from this molecule either by intrinsic feed phytase, microbial phytase or intestinal phytase (Abd El-Hack *et al.*, 2018). In plant seeds and grains the major storage form of phosphorus is phytic acid. The efficiency and the amount of endogenous phytase which is produced in the gastrointestinal tract (GIT) of poultry birds is not sufficient to hydrolyze the phytate present in feed stuff, so it reduces

the digestibility of nutrients which are phytate chelated and therefore reduces the bioavailability of phosphorus phytate is therefore considered as an anti-nutritional factor in animal feeds. To overcome this problem, inorganic or nonphytate phosphorus is added in the feed to meet the animal body requirements which increases the cost of production (Ravindran *et al.*, 1999). Dietary phytases are the enzymes that can produce a physiological effect on the digestion processes of proteins and carbohydrate by modifying endogenous secretions of different digestive enzymes and nutrient transportations in the gastrointestinal tract of chickens (Cowieson *et al.*, 2007). Non-starch polysaccharides (NSP), phytate, tannins and other anti-nutritional factors in cereal grains, seeds and their by-products reduce their digestibility and nutrient availability, and therefore their feeding value too (Annison *et al.*, 1995).

In animal bodies the second most occurring mineral is phosphorus and approximately 80 % of this mineral is found in ash, bone and teeth. Phosphorus with calcium helps in the maintenance and formation of bones in the body and 20 % of phosphorus which is not found in the skeletal muscles and tissues is widely distributed in the soft tissues and other body fluids inside the body, where it performs a variety of essential functions. Approximately, two-third of the total phosphorus is present in the form of

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phytate in those plants, which forms major constituents of poultry feeds and it is not available or very poorly utilized by monogastric animals and humans and (Viveros *et al.*, 2000).

## MATERIALS AND METHODS

### Experimental design

A total of 96 commercial day-old broiler chicks were procured from the local market and transferred to poultry shed at University of Veterinary and Animal Sciences, Lahore. The shed was fumigated and white washed before arrival of the chicks. Chicks were weighed and all birds were randomly divided into 4 groups (A, B, C and D), with four replicates in each (n=6 in each replicate). Chicks were raised in electrically heated battery brooders for 7 days. The chicks were raised as per standard managerial conditions and provided feed and water *ad libitum* up to 35 days of their age without supplementation of coccidiostats or antibiotics.

**Table I.- Basal feed formula.**

Ingredients	Volume (gm/kg)
Wheat bran	250.0
Yellow corn	375.0
Soybean meal (48% protein)	285.0
Canola oil	50.0
Dicalcium phosphate <sup>a</sup>	6.0
Limestone	17.0
Vitamins and minerals premix <sup>b</sup>	5.0
Sodium chloride	2.2
Choline chloride 70%	1.0
DL- Methionine	2.0
L- Lysine HCl	0.3
Coccidiostat	1.0
Growth promotant	0.5
Chromic oxide	5.0
ME (Kcal/kg)	2910

<sup>a</sup>Dicalcium phosphate 220 g/kg Ca and 187 g/kg phosphorous. <sup>b</sup>Supplied per kg of diet: vitamin A (retinyl palmitate + retinyl acetate), 11000 IU; vitamin D, 2200 IU; vitamin E, 30 IU; riboflavin, 6.0 mg; thiamine, 1.5 mg; menadione, 2.0 mg; pyridoxine, 4.0 mg; niacin, 60.0 mg; vitamin B12, 0.02 mg; folic acid, 0.6 mg; pantothenic acid, 10.0 mg; biotin, 0.15 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 10.0 mg; manganese, 80.0 mg; iodine, 0.8 mg; selenium, 0.3 mg.

### Dietary treatments

A corn-soybean meal based diet contained 250g wheat bran/kg (Table I) offered to control group (group D), and the treatment groups, A, B, and C were offered

with same diet supplemented with various level phytase enzyme. Group D served as control group which was offered feed without phytase enzyme supplementation. Groups A, B and C were treatment groups and offered feed with phytase enzyme at the levels of 500FTU, 1000FTU, and 1500FTU per kg of feed respectively.

### Growth performance

Birds were weighed on very first day of experiment and then on weekly basis up to the end of the experiment to determine body weight gain. Daily feed offered and refused, of each replicate was also being recorded on daily basis. Data thus recorded regarding body weight and feed consumption was used to calculate feed conversion ratio (FCR) on weekly basis and then at the end of the experiment. Two birds from each replicate were slaughtered on day 35 to collect visceral organs. The weights of spleen, proventriculus, empty gizzard, heart, liver, and empty intestines were measured and presented as a percentage of the total body weight (ratio of weight of organ to body weight). Moreover, the length of intestinal tract was also measured.

### Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Data is presented as mean  $\pm$  S.E.M. The Kolmogorov Smirnov test was employed to test the normal distribution of the data. The data is analyzed using one-way analysis of variance (ANOVA, completely randomized design) and body weight and feed conversion ratio is analyzed using repeated measure ANOVA. The group differences are compared by the Duncan's Multiple Range Test (Duncan, 1955). Differences are considered significant at  $P < 0.05$ .

## RESULTS

### Zootechanical parameters

The results showed that weekly based feed intake and overall feed consumption did not change ( $p < 0.05$ ) in control and other treatment groups (Tables I, II). The results showed that weekly body weight gain of broilers were significant ( $p < 0.05$ ) in all weeks except in 2<sup>nd</sup> week and 5<sup>th</sup> week in which weight gain was not significant ( $p < 0.05$ ) in the control and phytase supplemented groups throughout the experimental period (Tables I, II). The results revealed that weekly and overall feed conversion ratio (FCR) was significant in the control and phytase supplemented groups (Tables I, II). Results revealed that the body weights of the phytase supplemented chicks were significantly different compared to the control group

(Tables I, II). Absolute weights of visceral organs of chicks have been presented in Table III. The results revealed that dietary supplementation of phytase enzyme have significant ( $p<0.05$ ) effect on weights of small intestine without digesta and increased weight of liver in group C as compared to control group. Greater increase was observed in group C when compared to control. Similarly absolute weight of spleen was tended to be higher in group C when compared to control of phytase enzyme have significant ( $p<0.05$ ) effect on weight of proventriculus in group C

where i have given its maximum dose as compared to control group. However no significant effect on weight of gizzard and relative live weights was observed in treated groups when compared to control. The absolute length of small intestine without digesta of broilers has been presented in Table III. The results revealed that dietary supplementation of phytase showed significant effect on lengths of small intestine. Significant ( $p<0.05$ ) increase in length of small intestine in Group C as compared to control group.

**Table II.- Effect of supplementation of different doses of phytase on growth performance in broilers on first five weeks.**

Parameters	Group A	Group B	Group C	Group D	P - value
<b>1<sup>st</sup> week</b>					
LBW (g)	144 <sup>ab</sup> ±0.8	147 <sup>a</sup> ±2.4	149 <sup>a</sup> ±2.6	140.5 <sup>b</sup> ±0.5	0.03
WG (g)	106 <sup>ab</sup> ±0.8	109 <sup>a</sup> ±2.4	111 <sup>a</sup> ±2.6	102.5 <sup>b</sup> ±0.5	0.03
FI (g)	129.7 <sup>ab</sup> ±0.6	129.08 <sup>ab</sup> ±0.9	128 <sup>b</sup> ±0.2	131.5 <sup>a</sup> ±1.2	0.05
FCR	1.2 <sup>ab</sup> ±0.01	1.2 <sup>b</sup> ±0.03	1.2 <sup>b</sup> ±0.03	1.3 <sup>a</sup> ±0.01	0.00
<b>2<sup>nd</sup> week</b>					
LBW (g)	334 <sup>bc</sup> ±3	341 <sup>ab</sup> ±1.3	348 <sup>a</sup> ±3.1	330 <sup>a</sup> ±323.1	0.00
WG (g)	349.2 <sup>a</sup> ±1.4	349.3 <sup>a</sup> ±1.4	349.1 <sup>a</sup> ±1.4	350.3 <sup>a</sup> ±0.8	0.91
FI (g)	190 <sup>a</sup> ±3.3	194 <sup>a</sup> ±3.4	199 <sup>a</sup> ±2.9	189.5 <sup>a</sup> ±2.5	0.15
FCR	1.8 <sup>a</sup> ±0.03	1.8 <sup>a</sup> ±0.03	1.8 <sup>a</sup> ±0.02	1.8 <sup>a</sup> ±1.8	0.15
<b>3<sup>rd</sup> week</b>					
LBW (g)	739 <sup>b</sup> ±2.1	750 <sup>a</sup> ±3.2	752.5 <sup>a</sup> ±3	715.3 <sup>c</sup> ±2.1	0.00
WG (g)	405 <sup>a</sup> ±2.7	409 <sup>a</sup> ±2.4	404.5 <sup>a</sup> ±4.5	385.3 <sup>b</sup> ±2.1	0.00
FI (g)	579.1±1 <sup>a</sup>	575.5±1 <sup>a</sup>	576±1.8 <sup>a</sup>	575.7±1.7 <sup>a</sup>	0.27
FCR	1.4 <sup>b</sup> ±0.00	1.4 <sup>b</sup> ±0.00	1.42 <sup>b</sup> ±0.01	1.5 <sup>a</sup> ±0.00	0.00
<b>4<sup>th</sup> week</b>					
LBW (g)	1169 <sup>b</sup> ±4.5	1172 <sup>b</sup> ±5.6	1188 <sup>a</sup> ±2.2	1143 <sup>c</sup> ±3.2	0.000
WG (g)	430 <sup>a</sup> ±5.7	422 <sup>a</sup> ±7.7	435.5 <sup>a</sup> ±5	427.8 <sup>a</sup> ±3.3	0.435
FI (g)	736.5 <sup>a</sup> ±2.3	735.6 <sup>a</sup> ±1.6	734.8 <sup>a</sup> ±2.1	737.7 <sup>a</sup> ±0.4	0.690
FCR	1.7 <sup>a</sup> ±0.03	1.7 <sup>a</sup> ±0.03	1.7 <sup>a</sup> ±0.02	1.7 <sup>a</sup> ±0.01	0.393
<b>5<sup>th</sup> week</b>					
LBW (g)	1700 <sup>b</sup> ±3.9	1712 <sup>b</sup> ±4.5	1729 <sup>a</sup> ±5.3	1680 <sup>c</sup> ±7.3	0.000
WG (g)	531 <sup>a</sup> ±6.7	540 <sup>a</sup> ±10.2	541 <sup>a</sup> ±4.8	537 <sup>a</sup> ±6.2	0.767
FI (g)	1009.1 <sup>a</sup> ±1.2	1010.8 <sup>a</sup> ±1.9	1009.2 <sup>a</sup> ±1.8	1003.1 <sup>b</sup> ±1.3	0.024
FCR	1.9 <sup>a</sup> ±0.02	1.9 <sup>a</sup> ±.03	1.9 <sup>a</sup> ±0.01	1.9 <sup>a</sup> ±0.02	0.745

LBW, live body weight; WG, weight gain; FI, feed intake; FCR, feed conversion ratio. Group A, 500FTU/kg feed; Group B, 1000FTU/kg feed; Group C, 1500FTU/kg feed; Group D, Control. Data was presented as Mean± S.E.M. Different superscripts <sup>a-b</sup> represent significant difference between the groups in a rows at  $P<0.05$ .

**Table III.- Effect of supplementation of different doses of phytase on relative body and organ weights in broilers.**

Parameters	Group A	Group B	Group C	Group D	P - value
LBW	1754.4 <sup>a</sup> ±61.3	1776.3 <sup>a</sup> ±55.0	1834.5 <sup>a</sup> ±20.7	1745.6 <sup>a</sup> ±72.4	0.676
Liver	45 <sup>b</sup> ±2.8	44.8 <sup>b</sup> ±2.01	52.1 <sup>a</sup> ±1.6	48.6 <sup>ab</sup> ±1.9	0.066
Heart	9.1 <sup>ab</sup> ±1.2	7.3 <sup>b</sup> ±0.5	9.8 <sup>a</sup> ±0.5	8.8 <sup>ab</sup> ±0.4	0.161
Spleen	2.1 <sup>a</sup> ±0.1	2.1 <sup>a</sup> ±0.2	2.6 <sup>a</sup> ±0.9	1.5 <sup>a</sup> ±0.1	0.518
EPT Int. Wt	48.9 <sup>b</sup> ±3.06	44.7 <sup>b</sup> ±1.7	58.8 <sup>a</sup> ±3.1	57.1 <sup>a</sup> ±3	0.003
Empty Proventriculus Wt	6.4 <sup>a</sup> ±0.4	7.4 <sup>a</sup> ±0.6	7.6 <sup>a</sup> ±0.5	6.3 <sup>a</sup> ±0.2	0.069
Empty Gizzard Wt	28.6 <sup>a</sup> ±1.5	25.3 <sup>a</sup> ±0.6	28 <sup>a</sup> ±2.01	24.9 <sup>a</sup> ±0.9	0.159
Intestinal length	63.9 <sup>b</sup> ±1.5	68.9 <sup>a</sup> ±1.5	72.1 <sup>a</sup> ±0.7	71.5 <sup>a</sup> ±0.8	0.000

Group A, 500FTU/kg feed; Group B, 1000FTU/kg feed; Group C, 1500FTU/kg feed; Group D, Control. Data was presented as Mean± S.E.M. Different superscripts<sup>a-b</sup> represent significant difference between the groups in a rows at P<0.05.

## DISCUSSION

Supplementation of phytase enzyme has become an effective tool to bring improvements in the bioavailability of phosphorus present in feed stuffs and also to minimize the environmental pollution of phosphorus which animals excrete to the environment. Soybean meal (*Glycine max*) acts as a major food item for humans and animals because of its high beneficial health and nutritional values. It is an important dietary source of protein, fat, vitamins, minerals and fiber. Soybean also is a source of many other valuable biologically active compounds such as phyto estrogens which has potentially high benefits for the health of human beings (Messina, 1999). Apart from this, there are other compounds which are present in soybean like phytate (anti nutritional factor) and inhibitors of trypsin that can act as negative nutritional factors and produces hindrance in protein digestibility and these chelated with other essential nutritional elements including Ca, Fe, and Zn hence reducing their availability in gut (Liener, 1994; Hurrell, 2003).

Phytases are the phosphor hydrolytic enzymes that are capable to start the stepwise removal of phosphate from the phytate. Due to soybean high protein quality it is extensively used as protein supplement in poultry feeds (Stahl *et al.*, 2003; Lei *et al.*, 1993; Boling *et al.*, 2000). Different techniques and strategies have been employed to bring useful improvements in its nutritional value which normally includes mixing with corn, and supplementing it with limiting amino acids (Liu *et al.*, 1997), supplementing with some enzymes or treating with some organic acids (Ravindram and Kornegay, 1993). Supplementations of exogenous phytases and carbohydrases increases the dietary utilization of all essential nutrients inside the body which on other way would be lost to the animals and

wasted to environment.

Furthermore, it is also well documented that benefits of phytase action are not restricted only to Ca and phosphorus release, but also include a better absorption of trace minerals also.

The results of our study revealed significantly higher differences (P < 0.05) in FCR, live body weight and no overall significant differences in, weight gain and feed consumption in experimental groups treated by phytase enzyme compared to the control group. In our study gradually increase in the body weight gain was observed in all groups but significantly no difference (P < 0.05) in the body weight gain of broilers was observed among control and phytase supplemented groups and feed conversion ratio was significant (P < 0.05) in phytase supplemented groups as compared to control. Similar findings were observed by Narasimha *et al.* (2013) and they reported that the body weight gain in broiler chickens fed with BD supplemented with NSP enzymes, synbiotics and phytase was significantly (P < 0.01) higher. Supplementation of NSP enzymes, synbiotics and phytase alone or in combination had significant effect on feed intake. Feed conversion ratio (1.86), which improved (P < 0.05) in comparison to basal diet (BD) (2.06) and standard diet (SD) (2.02), respectively. The cost of feeding was lower (P < 0.01) in BD. Addition of these feed additives to BD did not increase the feeding cost and was comparable to unsupplemented ones and lower (P < 0.01) than SD.

In our study the effect of supplementation of phytase on relative weights and lengths of visceral organs were observed. The results revealed that dietary supplementation of phytase have significant (P < 0.05) effect on the weight and length of empty small intestine in treatment groups when compared to control. There are no significant effects on relative weights of spleen, liver; heart, gizzard, and

proventriculus were observed in treated groups when compared to control.

*Statement of conflict of interest*

Authors have declared no conflict of interest.

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