

Original Research Article

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Influence of Microbial Phytase on Tibia Characteristics and Biochemistry in Blood Serum of Broiler Chickens

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ABSTRACT

Keywords

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Two hundred ten (210) of one day old commercial broiler chicks (VenCobb 500) were divided into five (T₀, T₁, T₂, T₃&T₄) groups of 42 birds in each with three replicate of 14 broilers in each with the aim to see the effect of microbial phytase enzyme on the tibia characteristics and biochemistry in blood serum of broilers. For said purpose, the birds of (T₁, T₂, T₃&T₄) groups were feed with basal diets having (250FTU, 500FTU, 750FTU & 1000FTU/kg) respectively, while T₀ group was feed without any phytase. The experiment was conducted for one day to 42 days and the birds of T₄ group having significantly (p<0.05) higher percentage of tibia characteristics such as dry weight, calcium, phosphorus and zinc of broiler chickens and also the biochemistry such as calcium and phosphorus in blood serum of broilers. Thus, the results show that the phytase enzyme having better effect on the tibia characteristics and biochemistry in blood serum of broilers. Aforementioned trial was carried out in summer seasons.

Introduction

Phytate is the major form of phosphorus found in cereal grains, beans and oilseed meals feed to poultry birds. Approximately 61–70% phosphorus found in poultry diet ingredients is in the form of phytate phosphorus. Some microorganisms do produce phytase, most frequently the *Aspergillus* genus. The monogastric animals like poultry birds are unable to utilize this phytate phosphorus, as they lack endogenous phytase, which necessitates in the addition of inorganic feed containing phosphates to poultry diets in order to meet the phosphorus

requirements of poultry (Yu *et al.*, 2004). Phytase in poultry diets improves gut health as indicated by reduced secretions from the gastrointestinal tract (GIT) which consequently improves the efficiency of utilization of energy. The main objective of this current review therefore is to determine the effect of dietary phytase feed additives on the broiler performance. Many studies showed that microbial phytase can be used to increase the availability of P and reduce its excretion (Waldroup *et al.*, 2000; Paik, 2003). Previous studies have mainly focused on the

utilization of 3-phytase (EC 3.1.3.8) derived from the *Aspergillus niger* (Panda *et al.*, 2007) as feed additives for broilers.

Phosphorus (P) is an essential mineral for growth and skeletal development in chickens and if deficient results in deleterious effects such as skeletal deformities and impaired metabolic processes and ultimately poor nutrient utilization and decreased performance (Scott *et al.*, 1982). As P plays an important role in the metabolism of primary nutrients, so its deficiency results in poor health and performance of chickens (Waldroup, 1999; Hatten *et al.*, 2001). Conventional vegetable feed sources have low (up to 30-40%) availability of P (Nelson, *et al.*, 1968; NRC, 1994), as P is in bound form phytate. Non-ruminants lack the endogenous enzyme for the hydrolysis of phytate, so there is a need for supplementation of inorganic P (di-calcium phosphate, DCP) to overcome the P deficiency (Sebastian *et al.*, 1998). Phytate binds amino acids by the formation of binary protein-phytate complexes in the gastrointestinal tract. These complexes are resistant to pepsin activity. Phytate has also been reported to promote the flow of endogenous amino acids (Thompson, 1988).

Materials and Methods

The trial was conducted in summer season at the poultry farm situated at Agriculture farm of the Institute of Agriculture Sciences, Banaras Hindu University, Varanasi-221005(India). Two hundred ten (210) of one day-old commercial broiler chicks (VenCobb-500) were divided into five groups (T₀, T₁, T₂, T₃&T₄) of 42 birds in each with fifteen replicate of 14 broilers in each. The birds of (T₁, T₂, T₃&T₄) groups were feed with basal diets having (250FTU, 500FTU, 750FTU & 1000FTU/kg) respectively, while T₀ group was feed without any phytase. The birds were kept under deep litter system. At the start of the experiment the broiler starter ration was

fed from one day to 21 days containing 23% CP (Crude protein) and 2900 Kcal/ME/kg of ration. Further broiler per-starter ration containing 20% CP (Crude protein) and 3000 Kcal/ME/kg was giving from 22 days to 42 days age chicks (Table 1). Self-compounded phytase enzyme was mixed at (250FTU, 500FTU, 750FTU & 1000FTU/kg) of broiler starter and broiler per-starter ration in (T₀, T₁, T₂, T₃ & T₄) groups respectively. The control group (T₀) was feed broiler starter and broiler per-starter ration without any phytase enzyme.

Determination of serum calcium and phosphorus

Calcium procedure

2.0 ml sample of clear blood serum was transferred by means of a pipette to a 250 ml Erlenmeyer flask. The sample was diluted to 50 ml with calcium-free distilled water. 0.4ml of 9 N KOH solutions and 1 drop of ammonium purpurated indicator was added. The 36 sample was rapidly titrated with constant swirling to the same purple end-point reached in a titrated blank (Elliott, 1952)

Phosphorus procedure

Three ml of a suitable dilute standard or sample was measured into a calibrated Klett tube. 0.5 ml of 1: 2 nitric acids was added and mixed by thorough shaking. Then 0.5 ml of 0.25 per cent ammonium vanadate solution was added and mixed by shaking. Finally 0.5 ml of 5 per cent ammonium molybdate, made to a volume of 5 ml with water, and mix by inversion. After the mixture has stood for 5 minutes, it was read in the calorimeter with the No. 42 (blue) filter. A blank was prepared with 3 ml. of 7.5 per cent trichloroacetic acid and the reagents added in the same amounts and ordered as the standard. In every case the calculations were based upon the reading after subtraction of the blank reading (Klett, 1952).

Bone mineral analysis

Six birds were selected based on the group mean weight for the bone mineral analysis. The tibia bones of the selected birds were removed carefully from the thigh and the fresh bones oven dried at 100°C to obtain a constant weight. The dry bones were ashed at 550°C for 6 hours to obtain the ash content of the bone. The bone ash was analyzed for calcium; phosphorus and zinc content using Atomic Absorption Spectrophotometry (AAS). Finally, the data analysis by programmer (SAS, 2004) software version 9.1 (SAS, Cary, NC) using general linear model (GLM) significant differences among treatment means are separated using C.D. method.

Results and Discussion

Towards the end of trial the tibia characteristics parameters dry ash (% of live wt.), Ash % minerals, (zinc, Calcium and Phosphorus % of ash) in right leg ash of broiler chickens under different treatments in 6th week which after slaughtered at summer season in. The effects of phytase supplementation at different levels on minerals content of tibia bone of birds are summarized in Table 2 showed significant difference ($p>0.05$) among treatments These results are supported with Ravindran *et al.*, (1995), Sebastian *et al.*, (1996b), Mitchell and

Edwards (1996), Qian *et al.*, (1997), Cabahug *et al.*, (1999) and P. K. Singh *et al.*,(2003) and, for Dry weight of tibia (% of live weight), phytase supplementation improved the weight of tibia and ash percentage. It was maximum at summer season (11.79% and 52.22%) respectively, in phytase supplemented diets having high phytase enzyme 1000FTU/kg. Phytase supplementation increased maximum of minerals (Ca, P and Zinc % of ash) (43.85, 61.80 and 20.69% of ash) respectively at summer season from the high phytase supplementation at T4 (1000FTU/kg).

The tibia ash concentrations of the birds feed on control and high phytase diets were significantly ($p>0.05$) different. The highest tibia ash were recorded in birds feed on the high phytase supplementation at T4 (1000FTU/kg). These results were similar with other reports Yan *et al.*, (2000), Viveros *et al.*, (2002), Shirley and Edwards (2003), Saima *et al.*, (2009) and walk *et al.*, (2014). The data revealed that application of phytase enzyme during summer season was combination showed significant difference ($P<0.05$) effect tibia characteristics parameters, dry ash (% of live wt.), Ash % minerals, (zinc, calcium and phosphorus % of ash) in right leg ash of broiler chickens at all the treatments of supplementation phytase enzyme compared with control treatment of observation during summer season.

Table.1 Chemical composition of broiler feed

No.	Specification	Percentage	
		Broiler Starter	Broiler per-Starter
1.	Crude protein	23.0	20.0
2.	Crude fiber	5.0	5.0
3.	Calcium	1.0	1.0
4.	Phosphorus	0.5	0.5
5.	Lysine	1.22	1.06
6.	Methionine	0.83	0.72
7.	M.E.	2900 kcal/kg	3000kcal/kg

Table.2 Effect of phytase enzyme supplementation on bone mineralization of broiler chickens in summer season

Parameters	Treatments					C.D. at 5%
	T0	T1	T2	T3	T4	
Tibia characteristics:						
Dry weight (% of live wt.)	7.69	8.48	8.97	10.50	11.19	3.5
Ash % of MD	47.36	49.86	50.65	50.95	53.22	5.9
Calcium (% of ash)	36.13	40.36	42.51	43.62	43.85	7.7
Phosphorus(% of ash)	39.81	46.50	55.48	58.21	61.80	22.0
Zinc (% of ash)	15.80	18.20	18.81	19.56	20.69	4.89

T0 (Control (Standard feed), T1 (Standard feed+ 250FTU/kg), T2 (Standard feed+ 500FTU/kg), T3 (Standard feed+ 750FTU/kg), T4 (Standard feed+ 1000FTU/kg).

Table.3 Effect phytase enzyme on biochemistry parameters minerals (Calcium and Phosphorus mg/dl) in blood serum of broiler chickens

Season	Parameters	Treatments					C.D. at 5%
		T0	T1	T2	T3	T4	
Summer season	Calcium mg/dL	8.15	9.48	10.33	10.80	11.33	3.18
	Phosphorus mg /dL	3.15	3.90	4.37	5.41	5.51	2.36

T0 (Control (Standard feed), T1 (Standard feed+ 250FTU/kg), T2 (Standard feed+ 500FTU/kg), T3 (Standard feed+ 750FTU/kg), T4 (Standard feed+ 1000FTU/kg).

The data revealed that application of phytase enzyme during summer season was combination showed significant difference ($P < 0.05$) on biochemistry parameters minerals (Calcium and Phosphorus mg/dl) in blood serum at all the treatments of supplementation phytase enzyme compared with control treatment of observation during summer season. Assay of biochemical indicator from blood serum is shown in Table 3. Compared with the T₀ control group broilers, the treatments supplemented phytase increased significant ($P < 0.05$) the serum phosphorus. Serum phosphorus m of treatment T₄ and T₃ broilers was higher than that of treatment T₂ and T₁ on broilers ($P < 0.05$). This was similar to results by Dendow *et al.*, (1995). There was no significant difference ($P > 0.05$) between treatments T₄ and T₃ on serum phosphorus of broilers. Serum calcium increased with treatments phytase increasing in diets compared with T₀ control. Serum calcium of treatment T₄ and T₃ broilers was higher than that of treatment T₂ and T₁ broilers ($P < 0.05$).

Regarding to the serum alkaline phosphatase, calcium of broilers receiving phytase, there was no significant difference ($P > 0.05$) between treatments T₄ and T₃ of broilers. Peng Ying *et al.*, (2011) and Mohamed *et al.*, (2014) got the same results when they studied the effects of phytase on the performance and calcium and phosphorus metabolism of AA broilers by supplementing phytase in low P diets. Serum calcium increased along with the used of serum phosphorus and the high concentration of serum was beneficial to the use of phytase.

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