ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.515.522

Research Article
The Responses of Exogenous Enzymes (Multi-Enzymes) on the Productivity and Stage of Production of Broilers Fed Vegetable-sourced Diets

[1]M. Abu-Tayyeb, [2]I. Jahan, [1]M.A. Hossain, [3]M.M. Hossain, [1]N. Akter and [1]B.K. Nath

[1]Department of Dairy and Poultry Science, CVASU, Khulshi, Chittagong 4225, Bangladesh
[2]Department of Botany, University of Chittagong, Chittagong 4331, Bangladesh
[3]Department of Livestock Services, OTI, Savar, Dhaka, Bangladesh

Abstract
Objective: This experimental study was conducted to investigate the effects of diets and enzyme supplementation on the live weight (LW), feed intake (FI), feed conversion ratio (FCR), viability, leg bone quality and meat yield traits of broilers. **Materials and Methods:** In a 2×2 factorial experiment, having two diet types [corn-soya meal (CSM) based and corn-wheat-soya (CWS) based] and two enzymes levels (with or without), Broiler chicks (n = 112; Ross308) were assigned to four dietary treatments (CSM-, CSM+, CWS- and CWS+) with four replicates, seven chicks per replicate in a CRD. The chicks were fed on the ready-made broiler diet up to 25 days, after that formulated diets were supplied the birds *ad libitum* up to 45 days. All the diets were iso-caloric and iso-nitrogenous in nature and supplemented with or without enzymes in mash form. **Results:** Data revealed that diets (CSM- and CWS-) had no significant effect ($p>0.05$) on the LW, FI and FCR of broilers but enzymes (CSM+ and CWS+) increased ($p<0.5$, $p<0.01$) the LW and FI on day 45. Enzyme, diet and their interaction had no influence ($p>0.05$) on the viability, latency-to-sit (LTS), gait-scoring (GS) and bone traits of broilers. Diet and their interaction had no influence ($p>0.05$) on the bone quality traits of broilers except for enzyme. The mineral concentration (Ca%) and other bone traits were increased ($p<0.05$) by enzymatic diets. The results of dressing percentage, drumstick weight, thigh weight, breast weight, wing weight, back weight, shank weight and neck weight percentage of broilers were unaffected ($p>0.05$) by enzyme, diet and their interaction. Enzyme and diet had no influence ($p>0.05$) over the breast weight but its interaction influenced ($p<0.05$) the breast weight (%) of birds. Giblet and back weights (%) were influenced ($p<0.05$) by the diet only. **Conclusion:** It could be concluded that broilers might respond positively to enzymatic diets at the later stage of production.

Key words: Bone quality, broiler, carcass traits, exogenous enzymes, latency to sit test, plant-based diets, poultry industry

Received: May 12, 2019 Accepted: July 17, 2019 Published: October 15, 2019

Citation: M. Abu-Tayyeb, I. Jahan, M.A. Hossain, M.M. Hossain, N. Akter and B.K. Nath, 2019. The responses of exogenous enzymes (multi-enzymes) on the productivity and stage of production of broilers fed vegetable-sourced diets. Int. J. Poult. Sci., 18: 515-522.

Corresponding Author: M.A. Hossain, Department of Dairy and Poultry Science, CVASU, Khulshi, Chittagong 4225, Bangladesh

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry is now emerging industry across the globe. The main goal of poultry integrators is to achieve optimum production with low investment. This trend is intensifying and putting force over the poultry geneticists and nutritionists to explore alternative policies for profitable poultry production. Different feeding strategies such as reduction of specific nutrient content with addition of various supplements in poultry diets have been potential to increase the productivity of broiler and reduction of feed cost. For the ban of animal by-products and indiscriminate uses of antibiotics in animal nutrition by European Union[1,2], poultry nutritionists and researchers are looking for alternative feed supplements to enhance poultry productivity. One such strategy of reducing feed cost, could be the uses of exogenous multi-enzymes in vegetable based diet for boosting poultry production.

Research focusing on the bird's endogenous enzymes[3] suggests that the young birds might be limited in the types and amounts of enzymes necessary to utilize a high carbohydrate and vegetable protein diet at an early stage, thus affecting nutrient digestibility. For this reason, there is a potentiality of using exogenous enzymes in the diets that might improve digestibility of the feeds as well as performance of the birds. Inclusion of enzymes to diets enables the birds to degrade anti-nutrient feed components, in particular, non-starch polysaccharides and phytate. Enzymes are found to promote the breakdown of starch, cell walls, storage proteins and anti-nutritional factors[4]. The purpose of adding exogenous enzymes to poultry feeds is to reduce the effects of the anti-nutritional factors of ingredients that are present in greater or lesser amounts in the diet. Non-nutritive feed additives such as exogenous enzymes and direct-fed microbial are a means to overcome the anti-nutritional components of soy products by improving bird performance[5,6,7], overall nutrient availability and potentially immune and intestinal health[8].

The use of commercial enzymes in poultry feeds has evolved greatly in the last few years, based on the efficacy of the new products and a better understanding of the relationship between enzyme activity and substrates available[9]. Recent studies have also supported that the addition of multiple enzyme preparation (Natuzyme) which has carbohydrase, protease and phytase activities, might be helpful in terms of growth performance and intestinal activity in diets based on all-vegetable feed ingredients[6,10,11].

Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore, pose no threat to the animal or the consumer. Enzymes not only enable livestock and poultry producers to economically use new feedstuffs but will also prove to be environmentally friendly, as they reduce the pollution associated with animal production. Since the use of antibiotics in feed will be restricted in the future, there will be growing interest in using enzyme as a bioactive compound for improving gut health. However, very limited research works have been done regarding enzyme supplement in all-vegetable diet and other nutrients. Enzyme supplement in vegetable diet might be economical or could have potential to improve the productivity and decrease production cost. Moreover, consumers might be benefitted to find organic, healthy and nutritious or quality poultry products, as the birds would be reared on vegetable sourced diets with enzyme only without using any antibiotics as growth promoters. Considering the above, the present study was undertaken to investigate the efficacy of enzymes on the gross responses, bone quality and meat yield traits of broilers fed enzyme-sourced diets.

MATERIALS AND METHODS

Enzyme preparation: A multiple enzyme preparation, Natuzyme (Bioproton Pty Ltd., Sunnybank, Australia) is a powder form composed of xylanase (10,000,000 unit kg^{-1}), cellulase (5,000,000 unit kg^{-1}), β-glucanase (1,000,000 unit kg^{-1}), pectinase (140,000 unit kg^{-1}) from *Trichoderma reesei* and *Trichoderma longibrachiatum*. It also contains protease (6,000,000 unit kg^{-1}) and phytase (500,000 unit kg^{-1}) from *Aspergillus niger* and α-amylase (1,800,000 unit kg^{-1}) from *bacillus subtilis*.

Animal husbandry: Day-old Ross male broiler chicks (n = 112; 46.34$\pm$0.27 g) were procured from a local commercial hatchery and used for conducting this trial from hatch to 45 days. The chicks were weighed initially and were immediately distributed randomly into four dietary treatments (details are shown below) in a 2$\times$2 factorial arrangement, each treatment replicated four times with 7 birds per replicate. The birds were reared in brooder cages, in an open-sided house up to 45 days weeks. For the first two days the birds were provided with a temperature of 33°C. The temperature was then gradually reduced by 1 or 2°C every 1 or 2 days until the chicks were 19 days old at which point the temperature was maintained at 24°C for the rest of the trial. Eighteen hours of lighting and 6 h darkness per day were provided throughout the trial period except for first week only and at this period continuous lighting (23 h light : 1 h darkness) program was maintained for the chicks.

Diet formulation: Four experimental diets (CSM-, CSM+, CWS- and CWS+) were formulated with maize, wheat, rich polish and vegetable oil as the main energy sources, along with soybean meal as the main protein sources (Table 1). The diets were formulated exclusively with the ingredients of plant origin with or without addition of multi-enzymes (Natuzyme). Two basal diets (CSM- and CWS-) were formulated with soybean meal along with basal grains including other stuffs as shown in Table 3. These were fed as such (CSM- and CWS-) or supplemented with microbial enzymes (Natuzyme, Bioproton Pty Ltd., Sunnybank, Australia) to create diets CSM+ and CWS+. Natuzyme is a multi-enzyme (containing amylase, xylanase, β-glucanase, cellulase, pectinase, protease and phytase activities) supplemented to the basal diet at the rate of 0.4 g kg^{-1}. All the test diets were iso-caloric and iso-nitrogenous in nature and supplemented with or without exogenous cocktail enzymes. Bird was fed ready-made starter broiler diet for the first 25 days and then finisher or formulated diet in mash form was used to feed the broilers entire trial period from the rest of the trial period (26-45 days).

Data and sample collection: Mortality of birds were recorded as it occurred, while body weight and feed intake were recorded weekly for the calculation of body weight gain and feed conversion ratio (FCR) was corrected for mortality. Viability was calculated from mortality of birds per replicate cage. On day 45, two birds were selected randomly per treatment and killed humanely to obtain the right tibia bone samples for measurements of leg bone characteristics (bone length, bone weight, width of bone) and mineral concentration (Ca and P). Meat yield (dressed yield, breast weight, thigh weight, drumstick weight, shank weight, giblet weight, back weight) characteristics were also recorded on the last day of experiment. Feed samples were also collected prior supplying the birds to assess the nutritive value of the feeds. Cost benefit was calculated at the last of the trial period.

Latency-to-sit (LTS) and gait-scoring tests: On day 35, leg bone development was assessed through LTS and gait-scoring tests. The LTS tests were performed according to the method of Berg and Sanotra[12], with some modifications. Birds were tested in pairs in a luke-warm water. The test is based on the fact that bodily contact with water is an aversive experience for broiler chickens. Standing time is therefore positively correlated to the strength of leg bone. Two birds per replicate were used to carry out this test. Plastic tub containing warm water (32°C) at a depth of 3 cm was used to carry on the test (Fig. 1). The water temperature was checked every 10 min with a thermometer to ensure the correct temperature was maintained and it was checked again before another batch of birds was tested. Hot or cold water was added to raise or lower the water temperature if needed. Two birds from each replicate were selected randomly from the

Table 1: Ingredient and nutrient composition of the test diets

	Dietary treatments			
Ingredients (%)	CSM-	CSM+	CWS-	CWS+
Maize	52.00	52.00	41.50	41.50
Wheat	0.00	0.00	11.00	11.00
Rich polish	12.50	12.50	12.30	12.30
Vegetable oil (palm)	3.40	3.40	3.80	3.80
Soybean meal	27.00	27.00	26.50	26.50
Limestone	2.50	2.50	2.50	2.50
DCP	1.50	1.50	1.50	1.50
Dl-methionine	0.15	0.15	0.15	0.15
L-lysine	0.20	0.20	0.16	0.16
Choline chloride	0.03	0.03	0.03	0.03
Salt (NaCl)	0.50	0.50	0.35	0.35
Enzyme (Natuzyme)	0.00	0.04	0.00	0.04
Vita-min-premix	0.25	0.25	0.20	0.20
Nutrient composition (%)-calculated value				
ME (MJ kg^{-1})	13.00	13.00	13.01	13.01
CP (%)	19.10	19.10	19.00	19.00
CF	4.36	4.36	4.27	4.27
EE	4.03	4.03	3.88	3.88
Ca	1.62	1.62	1.60	1.60
AvailableP	0.80	0.80	0.80	0.80

CSM- and CWS- refer to basal diets without supplementation of any enzymes, whereas CSM+ and CWS+denote diets supplemented with multi-enzymes (Natuzyme). CSM: Corn-soya based diet and CWS: Corn-wheat-soya based diet, Enzyme was added in the diet as per the recommendation of the manufacturer company

Table 2: Chemical analyses of the formulated diet

	Nutrients (%)				
Treatments	H_2O	DM	CP	CF	EE
CSM-	9.59	90.41	17.38	4.82	9.21
CSM+	9.66	90.34	17.68	5.00	9.02
CWS-	9.98	90.02	17.33	4.42	10.00
CWS+	9.71	90.29	17.33	5.00	10.48

Table 3: 3-point scoring system

Gait scores	Degree of impairment	Criterion
1	None	Bird can walk at least 1.5 meter with a balanced gait. Bird may appear ungainly but with little effect on function
2	Obvious impairment	Bird can walk at least 1.5 meter but with a clear limp or decidedly awkward gait
3	Severe impairment	Bird will not walk 1.5 meter. May shuffle on shanks or hocks with assistance of wings

Fig. 1: Latency-to-sit test

cage and put into the water tubs. The tubs were covered with mesh lids to prevent birds from flying out. The birds were observed and left to stand in the tubs of water for up to 10 min. The time elapsed before the birds first attempted to sit down in the water was recorded in seconds with the aid of a stopwatch. If any bird was still standing after 10 min, the test was terminated.

The gait-scoring test conducted followed the 3-point scoring system developed by Kestin *et al.*[13] and Webster *et al.*[14]. To conduct the test, two birds were randomly selected from each replicate group and allowed to walk freely on the floor. The birds were then scored by visual observation against a number of criteria as described in Table 3.

Samples processing and analyses: Feed: Four feed samples from finisher diets were collected from the formulated diets prior feeding the birds. The samples were processed by grinding with the help of mortar and pestle and then mixed thoroughly for lab analyses. About 500 g of each diet of starter and finisher were taken and sent to the lab for further analysis. Each analysis was done three times for each sample to minimize technical errors. The samples were tested for dry matter (DM %), moisture (%), crude Protein (CP%), crude fiber (CF%), ether extract (EE%) and ash using standard laboratory procedure[15]. Dry matter was determined by oven dry method. Crude protein was recovered by Kjeldahl process. Ether Extract was quantified by Soxhlet apparatus. Ash was measured by igniting the pre-ashing sample on a muffle furnace at a temperature of 600°C for four to 6 h. The feed analytical report is shown in Table 2.

Bone processing and characteristics : The collected tibia bones were boiled for 10 min in deionized water, to remove all the soft tissue and defatted. After that, bone characteristics such as bone length and head width were measured using digital callipers (Mitutoyo, Japan) and the weight was recorded. After taking the measurements of the bone traits, the samples were ground and put on a muffle furnace at a temperature of 600°C for 4 h to make ash. The bone ash weight of samples was recorded. After that, the ash was analyzed for bone mineral concentration (Ca and P only), by atomic absorption and spectrophotometry, respectively.

Statistical analyses: All data were subjected to analyze by the GLM procedure using Minitab statistical software[16]. The significance of differences between means was tested using the Duncan multiple-range test. Statistical significance was considered at $p \leq 0.05$.

RESULTS

The gross responses and livability of broiler chickens: The results of body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens fed vegetable based diet supplemented with exogenous multi-enzyme were shown below in Table 4. The data showed that diet and its interaction (Diet×Enzyme) had no significant ($p>0.05$) effect on the BW, FI and FCR of broiler chickens except for enzyme. Increased ($p<0.05$) BW and FI were found in the broilers fed diet supplemented with enzyme (CSM+, CWS+) compared to others diets (CSM-, CWS-) without affecting ($p>0.05$) FCR on

Table 4: Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of broiler fed vegetable based enzymatic diets from 25-45 days

		BW (g/b)			FI(g/b)			FCR		
Diets	Enzyme	D31	D38	D45	D31	D38	D45	D31	D38	D45
CSM	-	1811.30	2137.50^b	2672.000^b	900.750	1801.50	3114.80^b	2.010	2.1700	2.4100
	+	1838.80	2200.00^a	2837.500^a	868.750	1795.80	3207.50^a	1.910	2.1800	2.2100
CWS	-	1803.60	2103.00^b	2618.800^b	875.000	1643.00	2926.00^b	2.050	2.1400	2.3700
	+	1772.50	2252.80^a	2846.000^a	859.000	1916.00	3324.00^a	2.110	2.1400	2.2500
Pooled SEM		8.85	23.425	20.800	20.190	39.40	51.350	0.081	0.0815	0.0715
Level of significance										
Diets (A)		0.059	0.859	0.561	0.668	0.824	0.767	0.607	0.8010	0.9850
Enzymes (B)		0.921	0.043	0.010	0.563	0.118	0.031	0.720	0.9300	0.1900
A×B		0.124	0.370	0.508	0.846	0.100	0.151	0.468	0.9720	0.7580

Table 5: Latency-to-sit (LTS) and gait-scoring (GS) values of broiler chickens fed vegetable diets with or without addition of multi-enzymes during 35 days

		Parameters	
Diets	Enzymes	LTS	GS
CSM	-	9.220	1.380
	+	7.780	2.130
CWS	-	7.190	2.000
	+	7.670	1.750
Pooled SEM		0.483	0.117
Level of significance			
Diets (A)		0.483	0.117
Enzymes (B)		0.289	0.603
A×B		0.626	0.306

Data represent mean values of four replicate groups consisting of 7 birds per replicate during d25-45days, [a,b]Means bearing uncommon superscripts within a column are significantly different at *p<0.05 and **p<0.01, SEM: Pooled standard error of mean, CSM: Corn-soya meal based diet, CWS: Corn-wheat-soya based diet

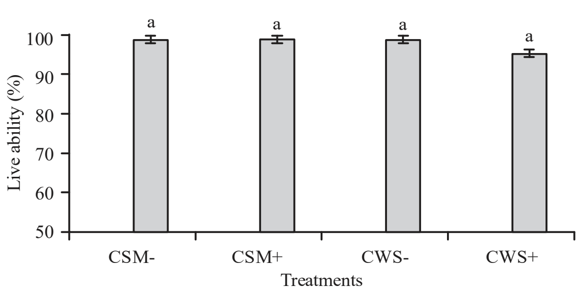

Fig. 2: Livability (%) of broilers fed enzyme supplemented diets from 25-45 days; Bars with similar letter are not significantly different (p>0.05)

day 45, respectively. Highest live weight and FI were recorded with the enzyme supplemented diets and the lowest live weight and FI were recorded at CWS diet without enzyme supplementation from 25-45 days. The diet, enzyme and their interaction had no influence (p>0.05) on FCR of broiler in this study.

Livability (%) of broiler chicken fed test diet: The livability (%) of broilers was not influenced (p>0.05) by diet, enzyme and its interaction, as shown in Fig. 2. It implies that the diet, supplemented diet and their interaction had a similar effect on the growth, development and mortality of broilers. The livability of broiler chickens was not affected by the supplementation of enzyme in the diet in the current study.

Latency-to-sit (LTS) and gait-scoring (GS) test: The results of LTS and GS tests denote that diet, enzymes and their interaction had no (p>0.05) effect on the LTS and GS of broiler chickens on day 35, as shown in Table 5.

Meat yield traits of broiler chickens: The results of meat yield characters *i.e* dressing yield, drumstick weight, thigh weight, breast weight, shank weight, giblet weight and back weights (%) of broilers chickens fed vegetable diets are shown in Table 6. The data show that diet, enzyme and its interaction (Diet×enzyme) had no significant effect (p>0.05) on these parameters such as dressing (%), drumstick weight (%), back weight (%), shank weight (%) and thigh weight (%)of broilers chickens. Diet has influenced (p<0.05) the giblet and back weight only. Interaction (Diet×enzyme) significantly (p<0.05) increased the breast weight of broiler chickens. Breast weight was poorly increased (p<0.097) by enzyme supplemented diets.

Bone (right tibia) characteristics and mineral concentration (Ca and P) of broiler chickens: The results of leg bone characteristics (e.g. bone length, bone weight, bone width and bone ash) and bone minerals (Ca, P) were shown in Table 7. Data shows that bone length (BL), bone weight (BW), bone width and bone ash (BA) were influenced by enzyme significantly (p<0.05). Diet and the interaction of diet×enzyme had no effect (p>0.05) on these bone traits such as bone length (BL), bone weight (BW), bone width and bone ash (BA) of broiler chickens. Diet and the interaction of diet×enzyme had no effect (p>0.05) on the Ca and P contents

Table 6: Meat yield traits (%) of broilers fed vegetable diets with or without enzymes on 45 days

		Traits								
Diets	Enzymes	Dressing (%)	Breast wt.	Drumstick wt.	Thigh wt.	Back wt.	Wing wt.	Giblet wt.	Shank wt.	Neck wt.
CSM	-	63.800	25.810	10.860	12.560	13.720^{a}	6.930	6.4000^{a}	3.820	2.930
	+	63.450	25.600	11.320	12.900	13.920^{a}	6.880	6.2400^{b}	3.830	3.000
CWS	-	60.480	24.440	10.280	12.820	12.510^{b}	6.220	6.1200^{b}	3.600	2.500
	+	62.910	25.940	11.000	12.850	12.830^{ab}	6.850	5.9000^{b}	3.880	2.790
Pooled SEM		1.128	0.150	0.233	0.205	0.166	0.129	0.0463	0.098	0.092
Significance level										
Diets (A)		0.445	0.162	0.417	0.811	0.026	0.359	0.0290	0.720	0.177
Enzymes (B)		0.671	0.097	0.258	0.679	0.484	0.205	0.1060	0.509	0.418
A×B		0.569	0.050	0.751	0.720	0.869	0.412	0.7800	0.508	0.623

Table 7: Leg bone traits of broilers fed vegetable diets with or without addition of multi-enzymes on 45 days

		Parameters					
Diets	Enzymes	Bone length (mm/b)	Bone wt. (g/b)	Bone width (mm/b)	Bone ash wt. (g/b)	Ca%	P%
CSM	-	65.4800^{b}	9.150^{b}	12.73^{b}	0.59^{b}	5.86^{b}	2.800
	+	68.0000^{a}	10.050^{a}	13.90^{a}	0.62^{a}	7.07^{a}	3.350
CWS	-	65.4300^{b}	9.200^{b}	13.15^{b}	0.57^{b}	5.20^{b}	2.400
	+	67.4000^{a}	9.950^{a}	13.46^{a}	0.61^{a}	6.34^{a}	3.150
Pooled SEM		0.299	0.078	0.105	0.004	0.197	0.151
Level of significance							
Diets (A)		0.613	0.881	0.964	0.144	0.172	0.377
Enzymes (B)		0.020	0.006	0.024	0.021	0.046	0.098
A×B		0.669	0.656	0.109	0.697	0.849	0.757

of tibia bone. Enzyme had ($p<0.05$) impact on the Ca contents of tibia bone and no impact ($p>0.05$) on P content of tibia bone.

DISCUSSION

The gross responses of broiler chickens fed vegetable based diets supplemented with exogenous enzyme: The gross responses of broiler in terms of feed intake (FI), body weight (BW) and feed conversion ratio (FCR) are the most important criteria for evaluating the growth performances of broilers. These criteria i.e., FI, BW and FCR etc., are considered as the key performance indicators (KPI) for assessing the broiler's potentiality in regard to growth responses. Results of the current study showed that, body weight of broiler was increased by the supplemental exogenous enzyme offered to the birds. The improved body weight of broilers fed on enzyme supplemented diet might be as a result of increased feed consumption. Further, the increased feed consumption of broilers fed on supplemented diets could be probably due to rapid growth rate of broiler chickens and therefore, more nutrients are required to ensure their faster growth. In addition, the increased feed intake of broilers might be due to higher fibre digestion as the vegetable feeds are rich sources of fibre. Initially, birds have filling effect in gut by fibre intake. After fibre digestion, chicks are able to increase feed intake to meet their nutrient requirements[17]. Moreover, the faster growth rate of broilers, might be due to greater feed intake.

Moreover, enzyme supplementation of diets might enhance the availability of certain nutrients, including trace minerals (e.g. Mn, Cu, Zn), which are known to promote greater feed intake of broiler chickens[18], enabling them to reach their full growth potential. It is reported that addition of microbial enzymes in diets might facilitate the broiler chicks to degrade the anti-nutritive factors such as NSP, in addition with promoting the breakdown of starch, cell walls and storage proteins[4]. Moreover, Lazaro *et al.*[19] reported that digest a viscosity might be reduced by exogenous enzymes, which could facilitate the improved contact between endogenous enzymes and nutrients, thereby improve the digestibility of the nutrients (amino acids).

Numerically lower FCR value was observed in the broilers fed diet supplemented with enzyme compared to diet without enzyme. It implies that birds fed on supplemental diets appears to be a bit more efficient in converting feed to meat. The improved feed consumption and body weight might be a result of better feed efficiency of broilers fed on supplemented diet, as is evinced in current study. Results of the current study are agreed with the findings of previous researchers[20,21,22]. Cowieson[23] and Zanella *et al.*[6] reported that exogenous enzymes can increase the feeding value of soy and other grain products, when fed to broilers, leading to increased growth performance and efficiency.

Livability of broiler chickens: The livability (%) of broilers was not influenced by diet, enzyme and its interaction, as observed form the current study. It can be assumed from the findings that for the growth and development of broiler chickens, basal diet, supplemented diet and their interaction had no effect on the livability of broiler chickens. Further, it implies that supplementation of exogenous enzyme in the broiler diets has no detrimental impact on the growth and survivability of the broiler chickens. Several studies demonstrated that enzyme could stimulate the natural immune system of poultry[24,25,26], which in turn could stimulate the similar livability of broiler chickens regardless of using enzymes in the diet. Hashemi and Davoodi[27] reported that enzymes improve the weight of immune organelles such as burse and spleen which can promote the immune situation and efficacy of health and livability. Freitas *et al.*[28] reported that using protease enzyme in the diet had no effect on the mortality of birds.

Meat yield traits of broiler chickens: The meat yield traits of broiler chickens were unaffected by enzyme supplemented diets in this study. This might be due to similar growth responses of broilers fed on enzyme supplemented diet. The breast weight was found to be a bit improved by enzyme supplemented diets. The results obtained contradict with the findings of previous researchers[11,29].

Bone (right tibia) characteristics and mineral concentration (Ca and P) of broiler chickens: Leg problems or disorders in broilers are an important welfare and economic issues for the poultry industry. Leg deformities in broilers due to genetic, nutritional and growth factors could result in decreased feed intake and body weight. Thus, feed optimization might be a strategy to decrease the severity of leg lesions in broilers[30].

In this study, all bone traits were influenced by dietary enzyme supplementation. The calcium percentage in bones was increased by supplementing enzymes, although LTS and GS tests depicted negligible influence. Dietary enzymes could help to release more nutrients from the feed for better growth and development of bones in broilers. Our results agree with the Sohail and Roland[31], who stated that enzyme (Phytase) supplementation significantly increases body weight, bone mineral content, bone density and livability of broilers.

According to Ravindran *et al.*[32], dietary phytase has been reported to increase mineral (Ca, P) retention and its concentration in serum and tibia of broilers. Phytate, being a strong acid, can form various salts with essential minerals, thus, reducing their solubilities and absorption[33]. When phytate is hydrolyzed by microbial phytase, it could release all mineral constituents, myo-inositol and inorganic phosphate[34].

CONCLUSION

It is concluded that broilers respond positively to the diets supplemented with exogenous enzymes in terms of increased feed intake, body weight and bone quality. Thus, enzyme supplementation might enhance nutrient availability and production potential of broilers affecting cost significantly.

REFERENCES

1. CEC., 2000. Council Decision of 4 December 2000 concerning certain protection measures with regard to transmissible spongiform encephalopathies and the feeding of animal protein. Official J. Eur. Communit., L306: 32-33.
2. Adil, S., T. Banday, G.A. Bhat, M.S. Mir and M. Rehman, 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology and serum biochemistry of broiler chicken. Vet. Med. Int. 10.4061/2010/479485.
3. Classen, H.L. and M.R. Bedford, 1999. The Use of Enzymes to Improve the Nutritive Value of Poultry Feeds. In: Recent Development in Poultry Nutrition, Wiseman, J. and P.C. Garnsworthy (Eds.) Nottingham University Press, Notingham, United Kingdom pp: 285-308.
4. Troche, C., X. Sun, A.P. McElroy, J. Remus and C.L. Novak, 2007. Supplementation of avizyme 1502 to corn-soybean meal-wheat diets fed to Turkey tom poults: The first fifty-six days of age. Poult. Sci., 86: 496-502.
5. Bedford, M.R., 1996. The effect of enzymes on digestion. J. Applied Poult. Res., 5: 370-378.
6. Zanella, I., N.K. Sakomura, F.G. Silversides, A. Fiqueirdo and M. Pack, 1999. Effect of enzyme supplementation of broiler diets based on corn and soybeans. Poult. Sci., 78: 561-568.
7. Knudsen, K.E.B., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol., 67: 319-338.
8. Zou, X.T., X.J. Qiao and Z.R. Xu, 2006. Effect of β-mannanase (Hemicell) on growth performance and immunity of broilers. Poult. Sci., 85: 2176-2179.
9. Choct, M., 2006. Enzymes for the feed industry: Past, present and future. World's Poult. Sci. J., 62: 5-16.
10. Douglas, M.W., C.M. Parson and M.R. Bedford, 2000. Effect of various soybean meal sources and Avizyme on chick growth performance and ideal digestible energy. J. Applied Poult. Res., 9: 74-80.

11. Cafe, M.B., C.A. Borges, C.A. Fritts and P.W. Waldroup, 2002. Avizyme improves performance of broilers fed corn-soybean meal-based diets. J. Applied Poult. Res., 11: 29-33.
12. Berg, C. and G.S. Sanotra, 2003. Can a modified latency to lie test be used to validate gait-scoring result in commercial broiler flocks? Anim. Welf., 12: 655-659.
13. Kestin, S.C., T.G. Kowles, A.E. Tinch and N.G. Gregory, 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec., 131: 190-194.
14. Webster, A.B., B.D. Fairchild, T.S. Cummings and P.A. Stayer, 2008. Validation of a three-point gait-scoring system for field assessment of walking ability of commercial broilers. J. Appl. Poult. Res., 17: 529-539.
15. AOAC., 2007. Official Methods of Analysis. 18th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
16. Minitab, 2000. Minitab Statistical Software User's Guide 2: Data Analysis and Quality Tools. Minitab Inc., State College, PA., USA.
17. Shrivastava, H.P., V.R. Sadagopan, T.S. Johri and S. Chand, 1981. Sunflower seed meal for poultry-A review. Indian Poult. Gaz., 65: 100-112.
18. Larbier, M.L., 1992. Metabolism of Water and Minerals. In: Nutrition and Feeding of Poultry, Larbier, M.L. (Eds.)., Nottingham University Press, United Kingdom pp: 101-118.
19. Lazaro, R., M.A. Latorre, P. Medel, M. Gracia and G.G. Mateos, 2004. Feeding regimen and enzyme supplementation to rye-based diets for broilers. Poult. Sci., 83: 152-160.
20. Hossain, M.A., 2013. Improving vegetable protein diets for broiler chickens. Ph.D. Thesis University of New England, Australia.
21. Hossain, M.A., M.M. Bhuiyan and P.A. Iji, 2015. Nutritive value of vegetable protein diets for broiler chickens and selection of diets containing different vegetable or animal proteins World's Poult. Sci. J., 71: 15-26.
22. Hossain, M.A., P.A. Iji and A.F. Islam, 2016. Gross responses and apparent ileal digestibility of amino acids and minerals in broiler chicken fed vegetable-based starter diets supplemented with microbial enzymes. Turk. J. Vet. Anim. Sci., 40: 583-589.
23. Cowieson, A.J., 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. J. Poult. Sci., 47: 1-7.
24. Lohakare, J.D., M.H. Ryu, T.W. Hahn, J.K. Lee and B.J. Chae, 2005. Effects of supplemental ascorbic acid on the performance and immunity of commercial broilers. J. Applied Poult. Res., 14: 10-19.
25. Houshmand, M., K. Azhar, I. Zulkifli, M.H. Bejo and A. Kamyab, 2012. Effects of non-antibiotic feed additives on performance, immunity and intestinal morphology of broilers fed different levels of protein. S. Afr. J. Anim. Sci., 42: 22-32.
26. Abbas, G., H.K. Sohail and R. Habib-ur, 2013. Effects of formic acid administration in the drinking water on production performance, egg quality and immune system in layers during hot season. Avian Biol. Res., 6: 227-232.
27. Hashemi, S.R. and H. Davoodi, 2010. Phytogenics as new class of feed additive in poultry industry. J. Anim. Vet. Adv., 9: 2295-2304.
28. Freitas, D.M., S.L. Vieira, C.R. Angel, A. Favero and A. Maiorka, 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. J. Applied Poult. Res., 20: 322-334.
29. Khan, S.H., R. Saradar and B. Siddique, 2006. Influence of enzymes on performances of broilers fed sunflower-corn based diets. Pak. Vet. J., 26: 109-114.
30. Williams, B., S. Solomon, D. Waddington, B. Thorp and C. Farquharson, 2000. Skeletal development in the meat-type chicken. Br. Poult. Sci., 41: 141-149.
31. Sohail, S.S. and D.A. Roland Sr., 1999. Influence of supplemental phytase on performance of broilers four to six weeks of age. Poult. Sci., 78: 550-555.
32. Ravindran, V., S. Cabahug, G. Ravindra, P.H. Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci., 41: 193-200.
33. Sandberg, A.S. and U. Svanberg, 1991. Phytate hydrolysis by phytase in cereals; effects on *in vitro* estimation of iron availability. J. Food Sci., 56: 1330-1333.
34. Wodzinski, R.J. and A.H.J. Ullah, 1996. Phytase. Adv. Applied Microbiol., 42: 263-303.