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Maximizing Performance with Safe Meat Production of Broiler by Feeding Enzyme Supplemented Antibiotic Free Diet

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ABSTRACT

The present work aimed at studying growth performance, carcass traits, and health status in broiler chicken-fed enzyme over a period of 4 weeks. A total of 300 day-old Lohmann broilers were randomly assigned to five treatment groups, each with 3 replicates (20). T1, T2, and T3 were provided as 0.05 %, 0.1 %, and 0.15 % of the enzyme. Whereas T4 and T5 were antibiotic and control, respectively. The results revealed no significant (P<0.05) difference in feed intake (T4 - 2182.50^{b}g and T2 - 2227.00 ^{ab}g) and live weight (T5 - 1897.50^{a} g and T1 - 1790.50^{b}). The highest survivability percent was found in the enzyme supplemented group (T1, T2 and T3 - 100%). Highest hemoglobin (T2 - 9.0 gm/dl), RBC (T2-3.9mill/cum), WBC (T1-14475 mill/cum), lymphocytes (T1 - 38.50%), Monocytes (T1 - 2.00%) PCV (39.96%), MCV (T1 - 88.58), MCH (T2 - 30.85 Pg) and MCHC (T2 - 32.80) were found highest in the enzyme-treated groups, which is an indication of good health. *E. coli* and *Salmonella sp*. The count was significantly (P<0.05) lower in birds fed a 0.15% enzyme supplemented diet and with a descending order of 0.1 % and 0.5% enzyme level. *Salmonella sp*. and *E. coli* count was also significantly (p<0.05) higher in birds fed control and antibiotic. The results of the study demonstrate the beneficial effects of supplementing enzymes on body weight gain and dressed yield in the treated groups in broiler chicken. An enzyme is, therefore, suggested to be used as an alternative to antibiotics on broiler chicken rations for higher profitability.

Keywords: Broiler, Enzyme, Safe Broiler, Microbiome, Gut Health.

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I. Introduction

The poultry industry is considered in nowadays to develop not only a better economy but a more health-friendly good quality protein source. To make this successful, research on safe broiler meat production is growing very fast. Although antibiotic has been beneficial for animal health and productivity, it has been also considered a double-edged sword. The massive use of these compounds has led to increased problems of antibiotic resistance [1]–[3] and the presence of antibiotics residues in feed and environment [3], [4] compromising human and animal health [6]. Hence, the identification and development of new and effective alternatives to antibiotics will help to produce health-friendly broiler meat.

In developed countries enzyme is used in the diet of humans, livestock, and poultry as a beneficial product. Thus, enzymes could be incorporated in poultry feed instead of antibiotics in order to stimulate or promote effective use of feed nutrients which results in a more rapid gain, higher production, and better feed efficiency. Moreover, Enzyme contains active substances that can improve digestion and metabolism and possess bacterial and immune-stimulant activities.

Exogenous enzymes are used to meet the lack of endogenous enzymes which are necessary for the digestion of certain type of nutrients in various feed stuff or hydrolysis for anti-nutritional factors present in the feedstuffs. By the use of an exogenous enzyme, the proportion of lactic and organic acids and VFAs concentration was increased and ammonia production was decreased [7]. The increased VFA concentration helps the hydrolysis of NSP and supports the growth of beneficial bacteria in the gut of the broilers. The exogenous enzymes modulate the gut microbiota of birds which may affect the health of the birds and the extent of digestion accomplished by the host [8]. Several authors have upkeep the thought that by application of enzymes production performances can be improved up to 10 %, [8]–[10] whereas some scientist does not support the positive effect of enzymes [11]–[13]. The obviously positive effect of these additives depends on the quantity and quality of feeds included in the mixture, used level of energy, type of enzymes, as well as fattening conditions [14]. This research was aim to investigate the profitable level of enzyme dose and

alternative antibiotics by improving the favorable microbiota to produce a safe broiler for the consumer.

II. Methodology

A total of 300-day-old Lohmann broiler chicks were placed under the two brooders. At 0.05 % (minimum dose of the treatments) enzyme with drinking water was supplied and in another brooder without enzyme. After one week 180 chicks were selected from enzyme-treated brooders and distributed randomly in three dietary treatments of enzyme; another 120 chicks were selected from the non-enzyme treated brooder and distributed randomly in one treatment for antibiotic and control. Each treatment had three replications with 20 birds in each. Experimental treatments were given below:

T1: 0.05 % of the enzyme (0.05 g enzyme/1 liter of water)
T2: 0.1 % of the enzyme (0.1 g enzyme /1 liter of water)
T3: 0.15 % of the enzyme (0.15 g enzyme /1 liter of water)
T4: Basal Diets + Antibiotics
T5: Basal Diets/ Control

The ration was formulated and prepared on the farm premises.

*Supplied per kilogram of diet: Vitamin A 10,000 IU, Vitamin D_3 2,000 IU, Vitamin E 10mg, Vitamin K 20mg, Vitamin B_1 2 mg, Vitamin B_2 10mg, Vitamin B_3 15mg, Vitamin B_6 300 mg, Vitamin B_5 10mg, Vitamin B_8 5mg, Vitamin B_9 2500 mg.

**Supplied per kilogram of diet: Manganese 500 mg, Iron 250 mg, Iodine 10 mg, Zinc 600 mg, Copper 100 mg, Selenium 1 mg, and Cobalt 1 mg.

The poultry farm and all necessary equipment were washed and dried. The birds were placed in two electric brooders. After one hour of chick placement, the feed particles were given to eat on newspaper. The experimental broiler chicks were randomly distributed to the experimental treatments after the brooding period at 7 days of age maintaining enzyme and non - enzyme-treated groups. In each replication, one feeder and one drinker were placed.

Starter feed was given from 0 days - 14 days while the grower feed was offered from 15 day-28 days. Birds assigned to all treatments were subjected to identical managemental procedures. Pre-weighed feeds were given to all treatments. The feed leftovers were measured weekly and deducted from the total amount of feeds offered to calculate the actual feed consumption of the birds during the starter and grower stage. Electric ceiling fans were used to control the temperature and humidity. At 28 days of age, the randomly selected bird was slaughtered by making an incision at the throat with a sharp knife. The slaughtered bird was further weighed to have the percent of blood loss. After the completion of bleeding the feathered broiler was dipped in hot water (140°F-160°F) for 5-6 minutes. All the feathers were plucked and weighed. The whole carcass was dissected on the same day and the meat was separated from the carcass using the procedure of Jones (1984).

In the brooder, the height of litter was 2 inches and in replications, it was 3 inches. To maintain litter quality it was racked daily up to 21 days and afterward twice daily up to 28 days. Required new litter was used over old litter if it seemed moist.

Only 3 vaccines were used within 28 days. The day-old chicks were vaccinated with a composite dose of vaccine against infectious bronchitis (IB) and Newcastle disease during placement in the brooders. On the day of the 9th and 18^{th,} the chicks were vaccinated against Gumboro disease. The medications were Vitamin-ADE, Vitamin -B complex, and Vitamin-C, Electrolytes, and Coxicure (Sulfaclozin Sodium) against coccidiosis in all treatment groups. Only 4 replications of the antibiotic group were treated with doxivet (Doxycycline Hyclate).

The parameters which were taken to determine the production performances are feed consumption, final live weight, feed conversion ratio, dressing percent, and mortality.

The hematological parameters were RBC, WBC, hemoglobin, PCV, MCH, MCV, MCHC, and counts of leukocytes. Liver, bursa, and spleen weights were taken to see the immunological status of the birds. During the collection of all samples, standard laboratory procedures were followed. The standard formula, procedures, and kits were used to find out desired data.

The data were subjected to statistical analysis by using Statistical Package for Social Sciences (SPSS) version 25. Differences between means were tested using Duncan's Multiple Comparison Test (DMCT), LSD, and significance was set at $P<0.05$.

III. Results

A. Production Performance

The production performance data such as feed consumption (FC), live weight (LW), feed conversion ratio (FCR), dressing percent (DP), and survivability percent (SP) are given in the following Table I.

TABLE I: Feed Ingredients and Nutrient Composition of Starter and Grower Ration

Ingredients	Starter Phase Day 1 to 14	Grower Phase Day 15 to 28
Corn,7.4 % CP	53.8	56.76
Soybean meal, 44.5 % CP	38.79	35.12
Soybean oil (%)	2.3	3.28
Oyster shell (%)	1.58	1.5
Sodium bicarbonate (%)	0.19	0.17
Dicalcium phosphate (%)	2.02	1.9
Salt (NaCl) (%)	0.2	0.23
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
DL-Methionine (%)	0.35	0.33
L-Lysine HCl (%)	0.2	0.16
L- Threonine (%)	0.07	0.05
Chemical Composition		
ME (Kcal/Kg)	2900	3000
CP (%)	22.1	20.69
Methionine (%)	0.65	0.9
Lysine (%)	1.26	1.23
Methionine + Cysteine (%)	0.9	0.82
Calcium (%)	0.92	0.84
Available phosphorus (%)	0.41	0.38

T_1 = Control, T_2 = Antibiotic, T_3= 0.5 % ENZ Supplementation, T_4 = 0.1% DSP Supplementation and T_5 =0.15 % DSP Supplementation.
Values are Mean (n=12) one-way ANOVA (SPSS, DMCT):
- Mean with different superscripts are significantly different ($P<0.05$).
- Mean within the same superscripts do not differ ($P>0.05$) significantly.
SE = Standard Error; NS = Non Significant; LSD = Least Significant Difference.

The highest feed was consumed by the 0.1 % enzyme-treated group (T4-2182.50[b]g) and the lowest also in Antibiotic treated group (T2-2227.00 [ab]g), but no significant (P>0.05) difference was found among enzyme-treated groups with control and antibiotic. The highest FC does not always result in good economic return as live weight.

No significant (P>0.05) difference was found in final live weight among enzyme-treated groups with control and antibiotic-treated group, but the highest LW was found in enzyme treated group (T5-1897.50[a] g) and the lowest in the control group (T1-1790.50[b]).

Although FCR data shows no significant (P>0.05) difference among treatments, however, enzyme-treated groups (T4-1.22) show better FCR than control groups (T3-1.33 and T1-1.25), respectively. Nagata e*t al.*, [10] reported that the protein and energy levels in diets containing phytase influenced feed intake, weight gain, and feed conversion rate of the broilers.

The highest DP was found in the enzyme-treated group (T5-77.75[a]) and the lowest in the antibiotic-treated group (T1-75.50 [b] %). But, DP of all the treatment groups was not affected (P>0.05) by the enzyme and antibiotic group compared with the control.

The highest survivability percent was found in enzyme supplemented group (T1-100%; T2-100%) and control group (T3-100%) which showed no significant (P>0.05) difference with the antibiotic group (T4 95.00[b] %). Enzymes increase, therefore, the availability of nutrients by breaking down specific chemical structures which endogenous digestive enzymes are not capable of breaking down or break down only partially. It reduces the visceral weight which increases the dressing percentage. The average weight of the following internal organs such as the liver, spleen, and bursa are presented in Table II.

The average liver weight of different treatment groups T1, T2, T3 and T4 were 53.62 g, 52.5g, 48.12 g and 49.62 g respectively, but the treatment groups were not affected significantly (P>0.05) either by enzyme or antibiotic in comparison with the control group.

The mean spleen weight of different treatment groups was 1.99 ± 0.005. Thus statistical difference was not significant (P>0.05). The experiment could improve the weight of the liver and spleen.

The mean bursa weight of different treatment groups was presented in Table III, but no significant (P>0.05) difference was found among different treatment groups. The antibiotic treatment group (T2-2.44 g) showed height weight, whereas the control group (T3-2.00 g) showed the lowest weight.

The hematological report of hemoglobin, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HCT/PCV, MCV, MCH, and MCHC of blood samples treated by chlorella and antibiotic is presented in Table IV.

TABLE II: WEIGHT OF INTERNAL ORGANS UNDER DIFFERENT TREATMENT

Treatment	Liver/bird (g)	Spleen/bird (g)	Bursa/bird (g)
T_1	53.62 [a]	1.98 [a]	2.00[b]
T_2	52.50 [a]	2.00 [a]	2.25[ab]
T_3	48.12 [a]	2.00 [a]	2.44[a]
T_4	48.12 [a]	2.00 [a]	2.00[b]
T_5	49.62[a]	2.00 [a]	2.18[ab]
Mean ± SE	50.00 ± 1.127	1.99 ± 0.005	2.17 ± 0.005
$LSD_{0.05}$	3.32[NS]	0.016[NS]	0.143*

T_1 = Control, T_2 = Antibiotic, T_3= 0.5 % ENZ Supplementation, T_4 = 0.1% DSP Supplementation and T_5 =0.15 % DSP Supplementation.
Values are Mean (n=12) one-way ANOVA (SPSS, DMCT):
- Mean with different superscripts are significantly different (P<0.05).
- Mean within the same superscripts do not differ (P>0.05) significantly.
SE = Standard Error; NS = Non Significant; LSD = Least Significant Difference.

B. Hematological Parameters

The hemoglobin, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HCT/PCV, MCV, MCH, and MCHC of different blood samples were not affected significantly (P>0.05) treated by enzyme and antibiotic in comparison with the control group. But, the highest hemoglobin (T2-9.0 gm/dl), RBC (T2-3.9 mill/cum), WBC (T1-14475 mill/cum), lymphocytes (T1-38.50%), Monocytes (T1-2.00%) PCV (39.96%), MCV(T1-88.58), MCH(T2-30.85 Pg) and MCHC (T2-32.80) were found in the enzyme-treated groups.

TABLE II: AVERAGE PRODUCTION PARAMETER OF DIFFERENT TREATMENTS

Treatment	FC /Bird (g)	Live Weight/bird (excluding average DOC weight 40g)(g)	FCR	Survivability %	DP %	Abdominal Fat (g)
T_1	2246.75 [ab]	1790.50[b]	1.25[a]	100.00[a]	75.50 [b]	39.38[a]
T_2	2227.00 [ab]	1816.25[b]	1.22 [a]	95.00[b]	75.75 [b]	28.00 [b]
T_3	2259.25[ab]	1830.00[ab]	1.23 [a]	100.00[a]	75.50[b]	26.12[b]
T_4	2182.50[b]	1786.00[b]	1.22 [a]	100.00[a]	76.50[ab]	24.38 [b]
T_5	2337.75[a]	1897.50[a]	1.23 [a]	100.00[a]	77.75[a]	22.00 [b]
Mean±SE	2250.65 ± 19.31	1824.05 ± 13.40	1.23 ± 0.005	99.00 ± 6.81	76.20 ± 0.313	27.98 ± 1.68
$LSD_{0.05}$	54.86*	34.64*	0.017[NS]	1.82*	o.866*	3.426*

TABLE IV: HEMATOLOGICAL PARAMETER OF DIFFERENT TREATMENTS

Treatment	Hb (Gm/dl)	RBC (mill/cum)	WBC (mill/cum)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosi-nophils (%)	HCT/PCV (%)	MCV (Fl)	MCH (Pg)	MCHC (g/dl)
T_1	8.20	3.80	14475	55.50	38.50	2.00	2.67	39.96	88.58	30.17	32.68
T_2	9.00	3.90	10375	58.25	33.25	1.50	3.67	37.20	87.90	30.85	32.80
T_3	8.40	3.50	11700	58.75	34.75	1.75	3.67	39.71	88.22	30.20	32.68
T_4	8.60	3.50	9066	60.25	34.50	2.00	2.67	37.71	88.21	30.17	32.77
T_5	8.70	3.50	9066	60.25	34.50	2.00	2.67	37.71	88.21	30.17	32.77
Mean ± SE	8.60 ± 0.35	3.70± 0.08	11404 ± 3064.49	58.18 ± 2.94	35.25 ± 2.74	2.33 ± 0.19	3.17 ± 1.62	38.64 ± 1.05	88.23 ± 0.51	30.33 ± 0.16	32.75 ± 0.15
$LSD_{(0.05)}$	1.08[NS]	0.25 [NS]	4231.79 [NS]	9.66 [NS]	8.64 [NS]	0.471 [NS]	1.49 [NS]	3.24 [NS]	1.69 [NS]	0.46 [NS]	0.48 [NS]

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C. Intestinal Microflora

The microbial load (total count, *E. coli* and *Salmonella sp*. for its beneficial effect) in broilers fed different levels of the dried enzyme is given in Table V, *E. coli* and *Salmonella sp*. count was significantly (P<0.05) lower in birds fed a 0.15 % enzyme supplemented diet and followed by 0.1 % and 0.5% enzyme level. *Salmonella sp*. and *E. coli* count was also significantly (p<0.05) higher in birds fed control and antibiotic.

TABLE V: INTESTINAL MICROFLORA PARAMETER OF DIFFERENT TREATMENTS

Treatment	*Salmonella sp.*	*Escherichia Coli*
T_1	3.95×10^{9b}	4.13×10^{9b}
T_2	7.40×10^{6a}	7.65×10^{6a}
T_3	4.52×10^{6b}	7.25×10^{6a}
T_4	4.93×10^{6b}	7.32×10^{6a}
T_5	$4.40\times10^{6\,b}$	5.30×10^{6b}
Mean ± SE	5.04±0.30	6.33±1.61
Level of Significance	*	*

T_1 = Control, T_2 = Antibiotic, T_3= 0.5 % ENZ Supplementation,
T_4 = 0.1% DSP Supplementation and T_5 =0.15 % DSP Supplementation.
Values are Mean (n=12) one-way ANOVA (SPSS, DMCT):
- Mean with different superscripts are significantly different (P<0.05).
- Mean within the same superscripts do not differ (P>0.05) significantly.
SE = Standard Error; NS = Non Significant; LSD = Least Significant Difference.

IV. DISCUSSION

A. Production Performance

The highest FC at T4 (2182.50^{b} g) and lowest in T2 group (2227.00 ^{ab} g), but no significant (P>0.05) differences were found. Similar findings were reported by [15] the results showed that several enzyme-based supplements added into the diets of broiler chicks did not affect feed intake.

No significant (P>0.05) difference was found in final live weight. However, the highest LW was found in the enzyme treated group (T5-1897.50^{a} g) and the lowest in the control group (T1-1790.50^{b}).

Similar results were obtained by Sun It has been shown that phytase could improve the availability of phytate P, total P, some other minerals, and amino acids [16], using a low-phosphorus corn-soybean meal diet, reported that the inclusion of a novel microbial phytase into the diet greatly increased ileal phytase P and total P absorption. Many scientists support that by the use of enzymes broiler production performances can be improved up to 10% [17], whereas in some papers the positive effect of enzymes wasn't registered [18]–[19]. The obviously positive effect of these additives depends on the quantity and quality of feeds included in the mixture, used level of energy and type of enzymes, as well as environmental conditions [20].

Although FCR data shows no significant (P>0.05) difference among treatments, however enzyme-treated groups (T4- .22) shows better FCR than control groups (T3-1.33 and T1-1.25), respectively. These results support that the use of exogenous enzymes individually or in combination improves dietary nutrient utilization, resulting in more uniform animal performance [21]–[23].

The highest DP was found in the enzyme-treated group (T5-77.75^{a}) and the lowest in the antibiotic-treated group (T1-75.50^{b}%). But, DP of all the treatment groups was not affected (P>0.05) by the enzyme and antibiotic group compared with the control [24]. studying the efficacy of enzymes in broiler diets, found that the addition of phytase, amylase, xylanase, and protease in diets with reduced metabolizable energy, calcium, and phosphorus content, promoted similar feed intake and weight gain to a diet with adequate nutrient levels. According to the authors, enzyme blends can be added to diets with reduced nutrient levels aiming to maintain broiler dressing percentage.

The highest survivability percent was found in the enzyme supplemented group (T1-100%; T2-100%) and control group (T3-100%) which showed no significant (P>0.05) difference from the antibiotic group (T4 95.00^{b} %). Enzymes increase, therefore, the availability of nutrients by breaking down specific chemical structures which endogenous digestive enzymes are not capable of breaking down or breaking down only partially. It reduces the visceral weight which increases the dressing percentage. According to [25], the purpose of adding exogenous enzymes to non-ruminant feeds is to reduce the effects of the antinutritional factors of ingredients that are present in greater or lesser amounts in the diet.

The average liver weight of different treatment groups T1, T2, T3, and T4 were 53.62 g, 52.5 g, 48.12 g, and 49.62 g, respectively, but the treatment groups were not affected significantly (P>0.05) either by enzyme or antibiotic in comparison with the control group.

Though these results are not fully supported by [26]. These authors observed that phytic acid is a potent chelating agent. Its negative charges react with the positive charges of some amino acids (lysine, arginine, histidine), some proteins (including those involved in protein digestion, such as pepsin and trypsin), and carbohydrates (α-amylase), forming insoluble complexes, thereby reducing their availability and digestibility and consequently affecting organ biometry.

The mean spleen weight of different treatment groups was 1.99 ± 0.005. Thus statistical difference was not significant (P>0.05). The experiment could improve the weight of the liver and spleen. However, it was reported [27] that the addition of phytase to the low-P diet of corn-soybean meal did not improve the weight of the heart, liver, and spleen. [28] conducted the experiment using the diet supplemented phytase (500 FTU/kg) to adjust the available P and Ca percentage according to BASF and got a similar result.

B. Hematological Parameters

The hemoglobin, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HCT/PCV, MCV, MCH, and MCHC of different blood samples were not affected significantly (P>0.05) treated by enzyme and antibiotic in comparison with control group. But, the highest hemoglobin (T2-9.0 gm/dl), RBC (T2-3.9 mill/cum), WBC (T1-14475 mill/cum), lymphocytes (T1-38.50 %), Monocytes (T1-2.00 %) PCV (39.96 %), MCV (T1-88.58), MCH (T2-30.85 Pg) and MCHC (T2-32.80) were found in the enzyme-treated groups, which is an indication of good health. The findings of the present study are in line with the results of [29].

C. Intestinal Microflora

The microbial load (total count, *E. coli* and *Salmonella sp*. for its beneficial effect) in broilers fed different levels of the dried enzyme is given in Table V, *E. coli* and *Salmonella sp*. count was significantly (P<0.05) lower in birds fed 0.15 %

enzyme supplemented diet and followed by 0.1 % and 0.5 % enzyme level. *Salmonella sp.* and *E. coli* count was also significantly ($p<0.05$) higher in birds fed control and antibiotic. These results are in accordance with the earlier findings of [30] and [31]. In addition, the results of the current study support the results of [32] and [35] as well as who found that enzyme is useful for the beneficial intestinal flora.

V. Recommendation

In developed countries, people use enzymes in the diet of humans, livestock, and poultry as supplements. It is now commercially available in Bangladesh. To make the ration cost-effective (as the enzyme is very costly) considering that limitation only 0.1 % and 0.15 % levels were used in the ration. The enzyme could be used as an alternative to antibiotics in broiler ration. The study, therefore, recommends conducting a field trial on a commercial poultry farm to fix the inclusion level of the composite enzyme.

VI. Conclusion

Broiler chicken treated enzyme showed no significant ($P>0.05$) difference with antibiotic and control groups in feed consumption (FC), live weight (LW), feed conversion ratio (FCR), dressing percent (DP), and survivability. But, the highest LW and better FCR were found in enzyme-treated groups (T3, T4, and T5). Similarly better DP was found in enzyme-treated groups and the control group compared with the antibiotic group. Enzyme-treated groups showed no significant ($P>0.05$) effect on the weight of the liver, spleen, gizzard, and bursa respectively compared to the antibiotic and control group. But in enzyme treated group liver weight was higher compared to antibiotics.

The hemoglobin, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HCT/PCV, MCV, MCH and MCHC of different blood sample were not affected significantly ($P>0.05$) treated by enzyme and antibiotic in comparison with control group. But, the highest hemoglobin, WBC, lymphocytes, Monocytes, PCV, MCV, MCH and MCHC were found in the enzyme treated groups. Above experimental data indicates that the inclusion of up to 0.15% enzyme in the basal diets of young broiler chicks might improve the development of the growth performances and improves BW gain, immune characteristics of broiler chickens. Moreover non beneficial microbes are also found less in highest enzyme supplemented diet. So, enzyme may be used in broiler ration in absence of antibiotic.

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Conflicts of Interest

The authors declare no conflict of interest.

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