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Study on the Effects of Dietary Enzymes and Probiotics on Intestinal Escherichia Coli of Broilers

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ABSTRACT

The purpose of this study was to explore the effects of enzymes, probiotics and their combinations on intestinal histopathological indexes of broilers. One hundred and fifty broilers were randomly assigned to five diet groups for 42 days. We measured the performance indicators of five treatment groups. On the 42nd day, 2 chickens per replicate were collected for microbe count, and about 5cm of the distal ileum was resected for histopathological examination. The results showed that the total heterotrophic count of the basal diet and the probiotics diet was similar to that of the other diets, but significantly ($P < 0.05$) higher than that of the other diets. The lactic acid bacteria count was the highest in the probiotics + enzyme feed, and the lowest in the probiotics (2.58×10^5 CFU/mL) and enzyme supplemented feed (1.45×10^5 CFU/mL). The total coliform number of antibiotic diet (14.12×10^5 CFU/mL) was significantly lower than that of other diets ($P < 0.05$). The total number of Escherichia coli was the highest in the antibiotic diet and the lowest in the probiotics + enzyme mixture diet. Micrograph of ileum under basal diet showed mucosal shedding and villi degeneration. In this experiment, the supplementation of probiotics, enzymes or their combinations had no significant effects on the growth response of broilers. However, the birds' gut integrity was improved. In poultry nutrition, a mixture of 0.4% probiotics plus 0.1% enzymes is recommended as an alternative to antibiotic growth promoters.

KEYWORDS

Broilers; Feed additives; Growth response; Histology.

1. Introduction

Concerns about the potential development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota led to the ban of antibiotics as growth promoters in poultry production (Mathur and Singh, 2005; Stanton, 2013). The National Agency for Food and Drug Administration and Control in Nigeria, has banned the use of antibiotics as a growth promoter and as a preventive measure

for mould proliferation in animal feed (NAFDAC, 2018). As a result, it became necessary to seek for viable alternatives that could enhance the natural defense mechanisms of animals and reduce the massive use of antibiotics. Alternatives like prebiotics (Heidarpour et al., 2011), synbiotics (Agboola et al., 2014), organic acids (Fernandes et al., 2014), phytobiotics (Gheisar and Kim, 2017), plant extracts (Kurekci et al., 2014), acidifiers (Markazi et al., 2019), etc. have been found to play an important role in improving growth performance, maintaining microbial balance and enhancing gut integrity in poultry (Hosseini et al., 2016). Probiotics are live microbial feed supplement that beneficially affect the host animal by improving its microbial intestinal balance (Fuller, 1989; Bidarkar et al., 2014). They improve a positive balance of the population of useful microbes in the intestinal flora by antagonistic action through the secretion of their metabolites such as bacteriocins, organic acids and hydrogen peroxide. In-feed enzymes are produced as fermentation products fromfungi and bacteria and help to break down certain components of the feed, such as non-starch polysaccharides (NSPs) and phytates, which are indigestible by the endogenous enzymes produced by birds (Khattak et al., 2006). Enzyme, break down the NSPs, decreases intestinal viscosity and eventually improve the digestibility of nutrients, thus, improving the gut integrity. It was therefore the objective of this study to determine the effect of dietary supplementation of probiotic, enzyme or their combination on growth response, intestinal microbial load and histopathological indices of broiler chicken.

2. Materials and Methods

This study was carried out at the poultry research unit of Teaching and Research Farm, University of Ibadan, Nigeria. The research site is situated geographically on the South-west zone of Nigeria.

Management of experimental birds

One hundred and fifty (150) one-day old unsexed Abor acre broiler chicks were used for the study. They were purchased from a reputable commercial hatchery farm in Ibadan, Oyo state. They were weighed, tagged and randomly allotted to 5 diets in a completely randomized design. Each diet had 5 replicates with 6 birds per pen and reared in two phases (starter phase, 0-21 and finisher phase, 22-42). Treatment 1 consists of the basal diet (negative control; NC): Treatment 2 was NC+0.1% antibiotic {oxytetracyclin} (positive control; PC), Treatment 3: NC+0.4% probiotic {Lactobacillus acidophilus + Saccharomyces cerevisiae}, Treatment 4: NC+0.1% enzyme {β-glucanase, phytase and organic acids} and Treatment 5: NC+0.4% probiotic+0.1% enzyme. Experimental diets for starter phase (Table 1) and finisher phase (Table 2) were formulated to meet the nutrient requirements of the birds according to the recommendation of NRC (1994).

Data collection

Feed intake was calculated as difference between amounts given and left over. The birds were weighed at the end of the starter and finisher phases and values were used to calculate body weight gain and feed conversion ratio.

Microbial analysis

On day 42, two birds per replicate were sacrificed and dissected and the digestive tracts were carefully excised. Digesta sample was harvested from two-third of ileal section between Meckel diverticum and lleo-caeco-colonic junction pooled according to replicates and frozen for further analysis. The digesta were mixed in a 10 ml prereduced salt medium (Holdeman et al., 1977) and serially diluted according to the procedure described by Engberg et al. (2004) to examine the count of Lactobacilli (Rogosa, CM 0627, incubated anaerobically 48 hours) and coliforms (Mackonkey, CM 0115, incubated aerobically 24 hours). Gut tissue sample was serially diluted from 10–7 to 10–3. From each dilution, 0.1 ml of the sample was plated onto the appropriate media. After incubation period of 48 hours, the plates were observed for bacterial growth and colonies were counted.

While the MRS plates were kept anaerobically in an anaerobic jar at a temperature 35°C for 48 hours. After 48 hours, the plates were observed for bacteria and colonies were counted.

Histopathological parameters

At the end of 6 weeks of the experiment, two birds from each replicate were selected and weighed. The birds were slaughtered and the digestive tracts were carefully excised. Intestinal samples were removed and then transferred into specimen bottles containing 10% formalin where normal hematoxylin and eosin standard procedures were performed according to the methods of Iji et al. (2001).

Proximate analysis

The proximate composition of the diets was determined according to the methods of AOAC (2000).

Statistical analysis

Data obtained were analyzed using ANOVA of statistical analysis system, SAS (2012). Means were separated using Duncan's multiple range test and tested at p=0.05 level of significance. The statistical design was:

$Yij = \mu + ti + eij;$

Where Yij for example, is the performance indices measured, μ is the overall mean, ti is the fixed effect of the treatments, and eij is the random error.

RESULTS Performance of broiler chickens fed diets supplemented with probiotic and enzyme at starter phase (0-21days) and finisher phase (22-42 days)

The results on the performance of birds at the starter and finisher phases are presented in Table 3. There were no significant differences observed in the final weight, feed intake, weight gain, and feed conversion ratio of birds on the dietary treatments.

Table 1. Gross composition (g/kg) of diets supplemented with probiotic and enzyme (starter phase)

Ingredient	**Negative control (NC) Basal diet**	**Positive control (PC) Antibiotic**	**NC + Probiotic**	**NC + Enzyme**	**NC + Probiotic + Enzyme**
Corn	566.00	565.00	562.00	565.00	561.00
Soyabean meal	370.00	370.00	370.00	370.00	370.00
fish meal	30.00	30.00	30.00	30.00	30.00
Soya Oil	8.00	8.00	8.00	8.00	8.00
Dicalcium phosphate	16.00	16.00	16.00	16.00	16.00
Broiler Premix	2.00	2.00	2.00	2.00	2.00
Limestone	4.00	4.00	4.00	4.00	4.00
Methionine	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
Table Salt	2.00	2.00	2.00	2.00	2.00
Antibiotic	0.00	1.00	0.00	0.00	0.00
Probiotic	0.00	0.00	4.00	0.00	4.00
Enzyme	0.00	0.00	0.00	1.00	1.00
TOTAL	1000.00	1000.00	1000.00	1000.00	1000.00

Calculated nutrient (g/kg)					
Crude protein	233.78	233.68	233.38	233.68	233.28
Energy ME, kcal/kg	3095.64	3092.21	3081.9	3092.21	3078.47
Ether extract	44.94	44.90	44.78	44.90	44.90
crude fibre	38.56	38.54	38.47	38.54	38.54
Calcium	7.72	7.72	7.72	7.72	7.72
Total phosphorus	7.51	7.50	7.50	7.50	7.50
Non-phytate P	4.10	4.10	4.10	4.10	4.10
Ca:NPP	1.88	1.88	1.88	1.88	1.88

Result on microbial population on diets supplemented with probiotic and enzyme is presented in Table 4. Total heterotrophic counts of birds fed control diet (61.92×105 cfu/ml) and probiotic diet (64.48×105 cfu/ml) were similar but significantly ($p<0.05$) higher than the antibiotic (45.16×105 cfu/ml), enzyme (47.70×105 cfu/ml) and probiotic + enzyme (47.06×105 cfu/ml) supplemented diets. The highest total Lactobacilli count (12.78×105 cfu/ml) was recorded in the mixture of probiotic + enzyme diet while least was observed in total Lactobacilli count of birds fed probiotic (2.58×105 cfu/ml) diet and enzyme supplemented diet (1.45×105 cfu/ml). Total coliform count of birds fed antibiotic diet was significantly (14.12×105 cfu/ml) lower than for those on other dietary treatments (basal diet: 27.24×105 cfu/ml; probiotic: 29.44×105 cfu/ml; enzyme: 23.50×105 cfu/ml; probiotic + enzyme: 27.90×105 cfu/ml respectively). Total Escherichia coli count was highest in birds fed antibiotic diet (28.98×105 cfu/ml) while least was observed in birds fed mixture of probiotic + enzyme supplemented diet (2.44×105 cfu/ml).

Plates 1 and 2 show photomicrographs of birds on basal diet (Treatment 1). Ileum of birds fed probiotic diet showed sloughed mucosa layer and degenerated villi, the lamina proprial showed degenerated tissues with moderate infiltration of inflammatory cells.

Plates 3 and 4 show photomicrographs of birds on antibiotic diet (Treatment 2). Ileum of birds placed on antibiotic diet showed normal mucosa layer with normal villi.

Plates 5 and 6 show photomicrographs of birds on probiotic diet (Treatment 3). Ileum of birds placed on probiotic diet showed normal lamina proprial, normal tissue and submucosal layer.

Table 2. Gross composition (g/kg) of diets supplemented with probiotic and enzyme (finisher phase)

Ingredient	**Negative control (NC) basal diet**	**Positive control (PC) antibiotic**	**NC + Probiotic**	**NC+ Enzyme**	**NC + Enzyme + Probiotic**
Corn	661.00	652.00	651.00	655.00	650.00
Soyabean meal	280.00	280.00	280.00	280.00	280.00
Fish meal	25.00	25.00	25.00	25.00	25.00
Soya Oil	8.00	8.00	8.00	8.00	8.00
Dicalcium phosphate	16.00	16.00	16.00	16.00	16.00
Broiler Premix	2.00	2.00	2.00	2.00	2.00
Limestone	4.00	10.00	8.00	7.00	8.00
Methionine	1.00	2.00	2.00	2.00	2.00
Lysine	1.00	2.00	2.00	2.00	2.00
Table Salt	2.00	2.00	2.00	2.00	2.00
Antibiotic	0.00	1.00	0.00	0.00	0.00
Probiotic	0.00	0.00	4.00	0.00	4.00
Enzyme	0.00	0.00	0.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00

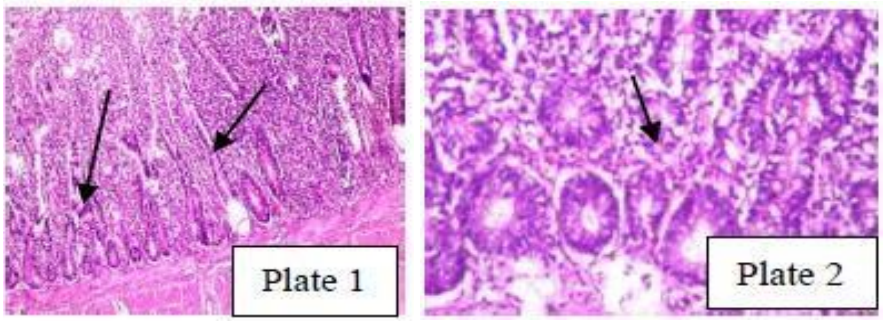
Calculated nutrient (g/kg) Crude protein	201.88	200.98	201.82	201.47	201.91
Energy ME, kcal/kg	3164.57	3133.66	3130.2	3143.97	3126.80
Ether extract	45.36	45.00	44.97	45.13	45.13
Crude fibre	34.31	34.11	34.52	34.30	34.30
Calcium g/kg	7.22	9.44	8.74	8.34	8.34
Total phosphorus	7.01	6.99	7.00	6.99	6.99
Non-phytate P	3.90	3.89	3.90	3.90	3.90
Ca:NPP	1.85	2.42	2.23	2.13	2.13

Table 3. Performance indices of broiler chickens fed diets supplemented with probiotic and enzyme at starter (d 0-21) and finisher (d22-42) phases

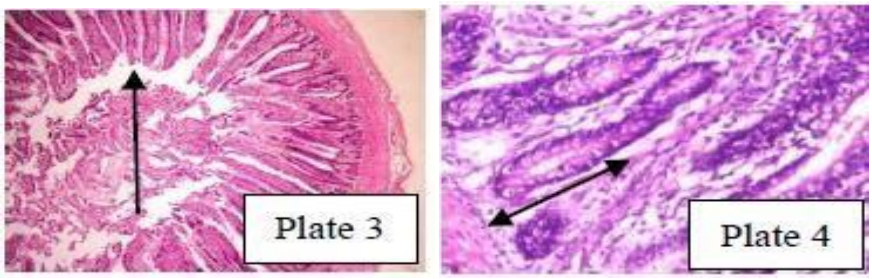
Parameter	Negative control basal diet	Positive control antibiotic	Probiotic	Enzyme	Probiotic + Enzyme	SEM	P value
Starter phase							
Initial weight (g/b)	41.50	41.92	41.2	41.62	40.9	0.26	0.78
Final weight (g/b)	590.10	597.12	564.66	528.12	545.18	10.15	0.19
Weight gain (g/b)	548.60	555.20	523.46	486.50	504.28	10.12	0.20
feed intake (g/b)	1286.1	1307.5	1482.7	1332.7	1474.7	47.53	0.54
feed intake (g/bird/day)	61.24	62.26	70.60	63.46	70.23	2.26	0.54
Feed conversion ratio	2.35	2.37	2.84	2.79	2.90	0.09	0.18
Finisher phase							
Initial weight	590.10	597.12	564.66	528.12	545.18	10.15	0.20
Final weight	1668.66	1660.94	1564.20	1519.22	1507.66	24.37	0.140
Weight gain (g/ bird/)	1078.56	1063.82	999.54	991.10	962.48	25.00	0.54
Feed intake (g/bird)	2340.10	2340.10	2584.2	2668.4	2223.8	84.94	0.45
Feed intake (g/bird/day)	111.43	111.43	105.90	127.07	105.90	4.05	0.45
Feed conversion ratio	2.18	2.21	2.56	2.75	2.42	0.10	0.40

Table 4. Microbial load (cfu/ml×105) of broiler chicken fed with diets supplemented with probiotic and enzyme

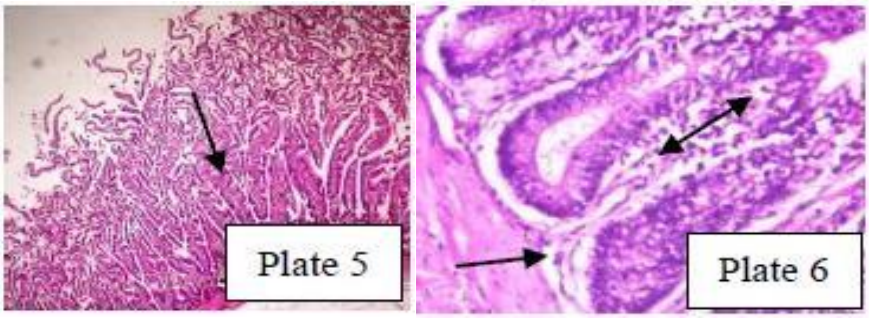
Parameter	Negative control basal diet	Positive control Antibiotic	Probiotic	Enzyme	Probiotic + Enzyme	SEM	P value
THC	61.92^{a}	45.16^{b}	64.48^{a}	47.70^{b}	47.06^{b}	1.70	0.0029
TLC	8.45^{b}	8.78^{b}	2.58^{c}	1.45^{c}	12.78^{a}	0.38	0.0001
TCC	27.24^{a}	14.12^{b}	29.44^{a}	23.50^{a}	27.90^{a}	1.61	0.0468
TEC	19.20^{c}	28.98^{a}	23.18^{b}	4.54^{d}	2.44^{e}	0.90	0.0001



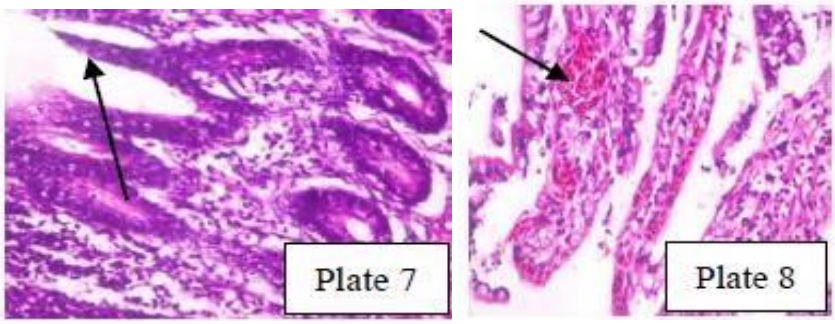
Plates 1 and 2. Photomicrographs of birds on basal diet (Treatment 1)



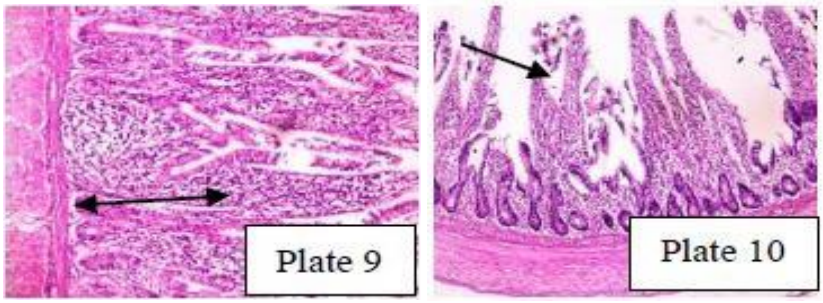
Plates 3 and 4. Photomicrographs of birds on antibiotic diet (Treatment 2)



Plates 5 and 6. Photomicrographs of birds on probiotic diet (Treatment 3)



Plates 7 and 8. Photomicrographs of birds on enzyme diet (Treatment 4)



Plates 9 and 10. Photomicrographs of birds on probiotic + enzyme (Treatment 5)

Plates 7 and 8 show photomicrographs of birds on enzyme diet (Treatment 4). Ileum of bird placed on enzyme supplemented diet showing normal mucosa layer with normal villi (Plate 7) but Plate 8 showed mucosa layer with moderately hemorrhagic villi.

Plates 9 and 10 show photomicrographs of birds on probiotic plus enzyme (Treatment 5). Ileum of birds placed on probiotic + enzyme: showing normal mucosa layer with normal villi (Plate 9) but Plate 10 showed poorly preserved mucosa layer with mildly sloughed villi.

3. Discussion

There were no remarkable differences observed in the feed intake, weight gain and feed conversion ratio of the birds on the experimental diets in both phases. These results corroborate the findings of Maiolino et al. (1992) who reported no observable effect of probiotic supplementation on performance of broilers. This was also supported by Agboola et al. (2014) who observed that the inclusion of probiotic and symbiotic in a corn-soyabean meal-based diet did not improve feed intake, feed conversion ratio and protein intake of turkey poults at the grower phase. In contrast, Miljkovic et al. (1997) asserted increased weight gain in birds upon feeding probiotic supplemented diet. According to Jin et al. (1998) and Patterson and Burkholder (2003), it was postulated that different results accrued in probiotic application to poultry diets probably depend on many factors, among which are species composition of probiotic, administration levels, application methods, overall diet composition, bird age and environmental factors. The result of this study is in agreement with observations of Loddi et al. (2000) and Willis and Reid (2008) who reported that supplementation of probiotics had no effect on the performance of broilers in any of the breeding phases. Fernandes et al. (2014) opined that birds fed alternative additives (prebiotic, probiotic, symbiotic or organic acid) had similar weight gain with those on antimicrobial product but were not different from birds on the control diet. Furthermore, Comert (2004) also reported that dietary mannan oligosaccharides and probiotic addition did not affect the feed intake of young turkeys from 0 to 8 weeks of age and Bronze turkeys from 7 to 21 weeks of age.

Contrary to the result of this study, Brenes et al. (1993) revealed that enzyme supplementation resulted in significant increase in body weights and feed conversion ratio in broiler chickens on barley-based diets up to six weeks. Also, Saleh et al. (2019) reported improved performance and nutrient digestibility in broiler chickens fed low-energy diets supplemented with mixture of dietary xylanase and arabinofuranosidase. According to Mehri et al. (2010), mannanase supplementation significantly reduced feed intake but did not influence body weight gain and feed conversion ratio in broiler chickens fed corn-soya diets. Similarly, dietary supplementation of enzyme cocktail of xylanase, amylase, and protease did not improve growth performance (weight gain and feed efficiency) in broiler chickens fed corn-soyabean diets for 21 days (Tiwari et al., 2010). Rexen (1981) however averred that effect of enzyme supplementation is more pronounced when the feed contains ingredients that are less-digestible. This statement was corroborated by Cozannet et al. (2017) and Aftab and Bedford (2018) who opined that diet composition is a key factor affecting the response to enzyme supplementation in poultry. This could be the reason why effect of enzyme supplementation was not pronounced on growth performance, in this study, because corn-soyabean meal diet was fed to the birds.

One benefit of using probiotics is to allow a numeric competitive advantage for beneficial intestinal microbes over the pathogenic microbes (Higgins et al., 2010). The result of the present study appears inconsistent. Birds on mixed probiotic-enzyme supplemented diets had significantly higher total Lactobacillus content compared to those on basal and antibiotic diets. It is however surprising that birds on probiotic and enzyme supplemented diets had very low counts of lactobacillus. Contrary to above finding, Biswas et al. (2018) reported an increase in the ileal and caecal Lactobacilli counts on days 21 and 42 in broiler chickens fed probiotic supplemented diets. In the present result, supplementation of mixed probiotic-enzyme and individual enzyme resulted in a lowered total E. coli count in comparison to the un-supplemented control. In agreement with the present finding, Mountzouris et al. (2010) reported reduction in the number of Enterobacteria in broiler chickens fed with a probiotic strain of L. reuteri. When Salim et al. (2013) fed broiler chicken a dietary supplement of directly-fed microbials, caeca lactobacillus content remained similar with birds on control (corn-soya) diet at 35 days, while cecal Escherichia coli content significantly decreased in broiler chickens fed directly-fed microbials. Mountzouris et al. (2009) showed that probiotic is effective at reducing the number of Salmonella enteritidis in broiler chickens. Rolfe (2000) suggested that probiotics exert their effects through competitive exclusion for adherence site on the gut, and for nutrients. This mechanism

might be responsible for the increased number of lactobacillus and simultaneous lowering of E. coli count in the gut of birds fed mixed enzyme-probiotic dietary supplement in the present study.

The efficiency at which digested nutrient are absorbed can be assessed using the histopathology of the intestine because it is the main site for nutrient absorption. This effect is determined by gross morphological features such as length and cross-sectional area of the duodenal, jejunal, ileal and caecal segments and by finer morphological features such as villus height and crypt depth as indicators of surface area of epithelium (Jin et al., 1998). Mucosa status and their microscopic structure may be good indicator of the response of intestinal tract to active substances present in feeds and in intestinal content (Viveros et al., 2011). In this study, histopathological changes observed in birds on negative control (basal diet) included sloughed mucosa layer and degenerated villi while other treatments showed normal submucosal and mucosa layers with normal villi and lamina propria showed normal tissues except for enzyme supplemented diet that showed mucosa layer with moderately hemorrhagic villi. Similar to the findings of Agboola et al. (2019), histopathological observation of broiler chickens on the control diet showed villi and hepatocellular atrophy but no lesions were found in the ileum of those on antibiotic supplemented diet. However, clinical symptoms ranging from villi atrophy, necrosis of the villi, loss of enterocyte, hepatocellular atrophy and focus of lymphoid aggregate in parenchyma of liver were observed in birds that received butyric acid supplemented diets. Unlike the pathological changes observed in the liver tissues of birds fed butyric supplemented diets, in this study, there was improved gut integrity because competitive exclusion of pathogenic bacteria that led to reduction in total Escherichia coli count with resultant increase in total Lactobacilli count was evident in birds fed with probiotic + enzyme supplemented diet. This was a reflection of improved submucosal and mucosa layers with normal villi which resulted in enhanced absorptive activity.

4. Conclusion

In this study, supplementation of diets with probiotic, enzyme or their combinations did not have remarkable influence on the growth response of broiler chicken. However, gut intergrity of birds was improved. Mixture of 0.4% probiotic + 0.1% enzyme is recommended to serve as subtitute to antibiotic growth promoter in poultry nutrition.

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