Effects of Commercial Probiotics on Productive and Physiological Performance of Broiler Chickens.

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ABSTRACT

A study was undertaken to test the effect of three commercially available probiotics on productive and physiological performance, immune organ weights, duodenum histomorphology and intestinal microbial examinations of broiler chickens. A total number of 240 one-week-old Cabb500 broilers were randomly divided into 4 groups with 3 replicates each group. 20 broiler chickens were placed in each replicate and the experiment lasted 6 weeks after start. Results revealed that supplementation of broiler diets with different types of probiotics have positive effects (numerically but insignificant) on both body weights and feed conversion ratio. Results of immunocompetent organ weights indicated significant differences in the weights of some lymphoid organs among the experimental groups. Results of total serum protein (STP), serum albumin (SA), serum globulin (SG) and serum immunoglobulin type M (SIgM) at 5 weeks of age revealed a significant ($P \leq 0.05$) increase in STP, SA, SG and SIgM of the treated groups compared to the control group. Addition of probiotics to broiler diets for a period of six weeks decrease significantly ($P \leq 0.05$) the thickness of both circular muscular and muscular mucosa layers compared to control group. The achieved results of villi height and villi width displayed that all groups of broiler chickens supplemented with probiotics were significantly ($P \leq 0.05$) increased in villi heights than the control group. Whereas group T4 showed significant ($P \leq 0.05$) increase in villi width than all other groups including control one. Probiotic treatments decreased clearly E.coli count, protous and shegella counts, and Klebsella and Enterobacter microorganism in broiler chicken intestine. On the other hand, supplementation of different types of probiotics in broiler chicken diets had a good effect to improve the microflora content in broiler intestine.

Keywords: Probiotics, Broiler chicken, Immune organs, Microbial contents, histomorphology.

INTRODUCTION

It is well recognized that gastrointestinal normal flora plays an important role in the health and performance of poultry. Worldwide, antibiotics have been used for decades in animal production at low levels of inclusion to enhance growth performance in farm animals. However, due to the emergence of microbes resistant to antibiotics used to treat infections in humans and animals, the Europe Union Commission decided to phase out and ultimately ban the marketing and inclusion of antibiotics as growth promoters in animal diets. This ban became effective on January 1, 2006 [17]. Consequently the focus in research has switched to feed additives as alternatives to antibiotics in poultry diets, apparently primarily to ensure gut health. Several possible mechanisms have been suggested such as altering of the gut pH, maintaining protective gut mucins, selecting beneficial intestinal organisms or ones antagonistic to pathogens, enhancing fermentation acids, enhancing nutrient uptake or increasing the humoral immune response [27]. These alternative feed additives include products such as enzymes, herbal products, microflora enhancers, immunomodulators, organic acids, probiotics, prebiotics or combinations of these products. Probiotic is a culture of live microorganisms (individual or groups microorganism) that can manipulate and maintain a beneficial microflora in the gut and can positively affect the animal by stimulating the activity and growth of beneficial native bacteria in the gastrointestinal tract and eliminate the pathogenic ones [20]. Certain species of bacteria, fungi and yeasts belong to the group of probiotics. Existing probiotics can be classified into colonizing species (Lactobacillus sp, Enterococcus sp. and Streptococcus sp.) and free, noncolonizing species (Bacillus and Saccharomyces cerevisiae) [64].

Under certain conditions probiotics alter the intestinal microbiota and immune system to reduce colonization by pathogens [44]. Numerous studies in humans and animals have been conducted to assess the ability of probiotics to change the type and number of the microflora in the digestive tract [53,21]. Some investigations on probiotics and organic acids with broiler chicks indicated positive responses to dietary supplementation [39,37]. In addition, Yeo and Kim [60] observed significant...
improvements in daily body weight gain and feed intake in broiler chicks receiving probiotics.

On the other hand, no positive results could be established in application of probiotic preparations in fattening of broilers in studies by certain number of researchers [35,40]. Wishing to explain in a scientific way inconsistent results which they obtained in their studies, majority of authors concluded that the effect of probiotics depended on the combination of bacterial strains contained in the probiotic preparation, level of its inclusion in the mixture, composition of mixture, quality of chickens and conditions of the environment in the production facility [29,44].

The main objective of the present study was to determine the performance, small intestinal microbial flora and tissue morphology of broiler chickens fed three probiotic commercial preparations.

### Materials and Methods

Two hundred and forty-one day-old unsexed broiler chickens (Cobb 500) were gift from Misr Arab Poultry Company, weighed and randomly allocated in four experimental groups of 60 birds each. Subsequently, the chickens of each group were distributed in 3 replicates of 20 chickens per replicate. For the experimental period of six week the birds in each replicate were raised on wheat straw litter as bedding material on the concrete floor and each pen contained a single feeder and drinker.

The experimental design consisted of four dietary treatments: T1 (a basal diet unsupplemented control diet); T2 (basal diet plus a probiotic based on photosynthetic bacteria, lactic acid bacteria and yeast at a level of 3.0 ml/kg of feed); T3 (basal diet plus a probiotic based on lactobacillus Sporagnes at a level of 0.5 g/kg of feed) and T4 (basal diet plus a probiotic based on Bacillus subtilis nato 6*10^7 cfu/g, at a level of 1.0 g/kg of feed). The commercial probiotics were used according to the manufacturers’ instructions.

The broiler starter and grower basal diets were based on maize and soyabean meal. The chickens were fed a broiler starter diet from days 1 to 10, a broiler grower diet from days 11 to 22 and a broiler finishing diet from days 23 to 42. The composition of the experimental diets is shown in Table (1). Nutrient levels of the diets for broilers were based on the breeder recommendations Guide. All chickens had free access to feed and water. A photoperiod of 23 h light and 1 h dark was applied.

Performance data were recorded weekly during the periods from 1 to 42 days of age. Feed intake was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each experimental period. Body weight and body weight gain was calculated as the difference between the final and initial bird weights. Feed conversion ratio was calculated as the ratio between feed intake and body weight gain during each phase of the experimental period. Feed was removed from each pen 12 h prior to weight.

At the end of the experiment (sixth week), 24 birds (6 from each group), were randomly selected, fasted overnight before blood samples were collected via jugular vein in heparinized tubes to determine the blood profiles and serum was separated and stored frozen at –20°C until analyzed.

Table 1: Compositions of experimental diets of broiler Cobb500 chickens during the tested stages.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter diet (1 – 10 day)</th>
<th>Grower diet (11 – 22 day)</th>
<th>Finisher diet (23 – 42 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn 8.5%</td>
<td>62.05</td>
<td>62.6</td>
<td>64.55</td>
</tr>
<tr>
<td>Soybean meal 44%</td>
<td>24.15</td>
<td>26.01</td>
<td>23.28</td>
</tr>
<tr>
<td>Corn gluten meal 60%</td>
<td>9.1</td>
<td>4.58</td>
<td>4.58</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>0.15</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.14</td>
<td>2.1</td>
<td>2</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.4</td>
<td>1.24</td>
<td>1.11</td>
</tr>
<tr>
<td>Vitamins and minerals premix(^1)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.12</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>L-lysine-HCl</td>
<td>0.29</td>
<td>0.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Total (Kg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Calculated analysis (^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein%</td>
<td>21.01</td>
<td>19.01</td>
<td>18.01</td>
</tr>
<tr>
<td>ME: cal/Kg feed</td>
<td>2966.70</td>
<td>3082.96</td>
<td>3176.00</td>
</tr>
<tr>
<td>C/P ratio</td>
<td>142.16</td>
<td>162.18</td>
<td>176.35</td>
</tr>
<tr>
<td>Calcium%</td>
<td>1.04</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td>Available Ph.%</td>
<td>0.50</td>
<td>0.49</td>
<td>0.47</td>
</tr>
<tr>
<td>lysine%</td>
<td>1.20</td>
<td>1.11</td>
<td>1.06</td>
</tr>
<tr>
<td>Methionine%</td>
<td>0.52</td>
<td>0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>Methionine + Cystin%</td>
<td>0.89</td>
<td>0.85</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\(^1\)Each 3Kg of vitamin and minerals mixture contain: Vit. A 10.000.000 IU, Vit. D₃ 2.000.000 IU, Vit. E 10.000 mg, Vit. K₃ 1.000 mg Vit. B₁ 1.000 mg, Vit. B₂ 5.000 mg, Vit. B₆ 1.500 mg, Vit B₁₂ 10 mg, Niacin 20.000 mg, Pantothenic acid 10.000 mg, Folic acid 1.000 mg, Biotin 50 mg, Choline chloride 500.000 mg, Copper 4.000 mg, Iodine 300 mg, Iron 30.000 mg, Manganese 60.000 mg, Zinc 50.000 mg, Cobalt 100 mg and Selenium 100 mg.

\(^2\)According to NRC (1994).
Serum total protein was determined according to Weichselbaum, [54]. Albumin was measured according to Doumas, [15]. The globulin values were obtained by subtracting the values of albumin from the corresponding values of total proteins. Serum immunoglobulin type M (IgM) levels were analyzed by Radioimmunoassay (RIA) method using RIA kits (Amersham international Ltd. Amersham, United Kingdom) according to method described by Li et al, [32].

After blood sampling birds were slaughtered for immune organs tests. The whole intestinal content was collected for total enterobacteriaceae count according to ICMSF, [28]. Also, samples were taken from the small intestine (duodenum) and fixed in 10% formalin saline for twenty four hours for histomorphological investigation. The examination was done through the light electric microscope as described by Bancroft and Gamble, [5].

**Statistical analysis:**

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package [51]. All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan’s multiple range test [16] at 5% level of significant. Data were analyzed by one way method using the following model. $Y_{ij} = u + N_i + e_{ij}$ Where $Y_{ij}$ = the observed value, $u$ = population means, $N_i$ = the effect of treatment, $e_{ij}$ = the standard error.

**Results and Discussion**

**Effect of probiotics in broiler diets on body weight, feed intake and feed conversion ratio:**

The effects of probiotics of (T2), (T3) and (T4) supplementation in broiler chicken diets on body weight, weekly feed intake and feed conversion ratio of experimental birds during the experimental period are presented in Tables (2, 3, and 4). In general, the differences in body weight among the experimental groups were insignificant. However, supplementation of broiler diets with different types of probiotics leads to clear numerical increase in the body weights and improvement in feed conversion of treated groups compared to control group (Tables 2 and 4). These results revealed that the dietary treatments had no significant effect on the mentioned parameters, whilst, at the third week of experiment group fed 1.0g probiotic/kg feed (T3) and 1.0g probiotic/kg feed (T4) compared to the control group at week 6 of experiment. The improvement noticed in this study for the body weights and feed conversion of broiler groups treated with different types of probiotics may refer to some reasons, a) improvement occurred in values of globulin and immunoglobulin type M (IgM) of treated groups compared to control which may reflect the better health and immunity status of these birds as seen in Table (6); b) treated groups significantly ($P \leq 0.05$) reduced the thickness of circular muscular and mucosal layers of duodenum compared to control group which lead to easy and fast transport of feed elements from intestinal to blood stream; c) villi height and villi width were significantly ($P \leq 0.05$) higher in treated groups compared to the control group and villi space was lower in the treated groups compared to the control group, these results reflect that there were better absorption for these groups because they had more surface area of villi for absorption than that found in the control group Table (7).

The beneficial effect of microorganisms of probiotics on the studied parameters may attribute to the fact that, when probiotics administered through the digestive tract, they have a positive impact on the hosts health through its direct nutritional effect [44]. Banday and Risam, [6]; Cmiljanic et al, [12], have suggested that probiotic supplementation improved performance of broilers. The different mechanisms of probiotic action suggested are; nutritional effect by regulation of metabolic reactions produces toxic substances; stimulation of endogenous enzymes and by production of vitamins or antimicrobial substances [34]. Moreover, probiotics could act as bioregulator of the intestinal microflora and reinforcing the host natural defenses, through the sanitary effect by increasing the colonization resistance and stimulation of the immune response. Also, this effect can be attribute to the trophic effect of this product on the intestinal mucosa, because it increases villi height, particularly during the first 7 days of the chickens life [49].

At the fourth week the feed intake was significantly ($P \leq 0.05$) higher for the group fed 3ml probiotic/kg feed (T2) compared to other groups. While at fifth week T2 showed significant ($P \leq 0.05$) lower feed intake compared to other groups. Furthermore, at week six of experimental period both of control group (T1) and group (T4) had significantly ($P \leq 0.05$) recorded less feed intake compared to other experimental groups. These results may refer to the fact that probiotics have ability to improve the microbial ecology of the intestine, reduce passage rate of the digesta and improve the digestibility of amino acids [7]. Changes in mucosal architecture, changes in gut environment and increases in villi height have been reported to be due to the supplementation of probiotics [57,25].
**Effect of probiotics in broiler diets on immune organ weights:**

Impacts of probiotics supplementation in broiler diets on immune organ weights is presented in Table (5). The obtained results of immunocompetent organ weights indicate that there were significant (P ≤ 0.05) and non significant differences in the weights of some lymphoid organs between all experimental groups as shown in Table (5). There were significant (P≤ 0.05) increases in spleen of control group (T1) compared with other groups. In this respect, Ubosi et al. [52] reported that the size of the spleen of poultry species may be affected by genotype. The spleen performs many functions and it is the major organ involved in immune response to some antigens, it also acts as an immunologic filter of the blood and entraps foreign materials that are antigens from the bloodstream passing through the spleen [38]. Furthermore, the spleen provides microenvironment, which is needed for antigens presentation and concentrating them in the white pulps where T and B cells interactions lead to the formation of antibodies. Also, the spleen and bursa are the important lymphoid organs involved in the development and differentiation of T or B lymphocytes [55,31].

Bursa weight was significantly (P ≤ 0.05) higher in group T2 compared to other groups except control group there was no significant differences between T2 and T1 in bursa weights. The increase in the bursa weight found in T2 may led to that T2 was significantly (P ≤ 0.05) higher in IgM value at week 7 as recorded in Table (6) which reflect better immunity status of broilers group T2 compared with the control group. These results may suggest that feeding broiler chickens diet contained probiotic (T2) at level of 3ml/kg feed enhanced the development of bursa organ of broiler chickens. Ubosi et al. [52] observed that a chicken line selected for high response to sheep red blood cells (SRBC) had a larger bursa size than the line selected for low response. Furthermore, Zhang et al. [63] showed clear association between non-MHC gen and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines. The bursa of Fabricius plays a central role in the development and maturation of the antibody-producing B-lymphocyte and humoral antibody in birds [63,9].

On the other hand, the weights of the rest of immune organs such as thymus and cecal tonsil weights did not show any significant differences (P≤0.05) between experimental groups due to supplementation of probiotics in broiler chicken diets. In regards to this point, Makram et al. [36] reported that the size of lymphoid organ weights may not associated with higher immune response of chickens and found that the Hubbard broiler chickens had higher relative lymphoid organs weight followed by Arbor Acres compared to Cobb and Avian broilers while, the Cobb strain had a higher immune response when compared to other broiler strains [36].

**Effect of probiotics in broiler diets on some serum constituents:**

Effect of probiotics supplementation on serum total protein (STP), serum albumin (SA), serum globulin (SG) and serum immunoglobulin type M (SIgM) at week 5, 6 and 7 are presented in Table (6). Results of STP, SA, SG and SIgM at week 5 of experiment revealed that there were significant (P≤ 0.05) increase in STP, SA, SG and SIgM of treated groups compared with the control group. The increased concentration of STP, SA, SG and SIgM in the serum of broilers by inclusion of probiotics in the broiler diets indicated that the probiotics affected protein metabolism, which is consistent with the observation of enhanced the body weights of treated groups but the enhancement was not significant. Results of STP show that group T3 was significantly (P ≤ 0.05) higher in concentration of STP followed by group T4 compared to other groups. While group T4 was significantly (P≤0.05) higher in concentration of SA followed by group T2 compared to other groups. The results of SG and SIgM revealed that the probiotic treatments cause a significant (P ≤ 0.05) increase in the concentrations of SG and SIgM of broiler chickens at week 5. The same trend was found with those parameters at week 6 and 7 of broiler age while STP and SIgM were increased significantly (P ≤ 0.05) as the age of broiler chickens advanced.

Increment in STP concentration in treated groups as compared to the control group (Table 6) may be attributing to the hormonal regulation of protein metabolism, for example growth hormone increased the synthesis of cellular protein, glucocorticoids increased break down of most tissue proteins. The increasing of corticosterone hormone and glucocorticoids which are secreted by the adrenal cortex increased the quantity of protein in most tissues while decreased the amino acids concentration in the plasma, as well as decreased both liver protein and plasma proteins, or may be due to the decrease of thyroxin secretion, that thyroxin increases the rate of metabolism of all cells and, as a result indirectly affects protein metabolism [22,2]. In the present study it is believed that increasing STP may refer to that the rate of anabolism was higher than the rate of catabolism in broiler chicken bodies and this may the reason that treated groups had higher body weights than the control group (Table 2). In the process of protein anabolism and proteolysis, the serum protein level usually reflects the protein metabolism and immunity function situation in vivo. Serum total protein contains albumin and globulin, both of which were shown to reflect the hepatic protein metabolic status in response to dietary treatments in broiler chickens [31]. The increased...
serum concentration of SA and SG may be indicative of enhanced immune system as the serum concentration of SA and SG proteins are regarded as the direct reference to the body immune function [62]. In the current study, it has been found that dietary supplementation of probiotics significantly ($P \leq 0.05$) increased the serum total protein compared to the control treatment. This result implies that dietary supplementation of probiotics can improve whole body protein anabolism in growing broilers.

The obtained results show that serum immunoglobulin type M (SIgM) levels Table (6) were significantly ($P \leq 0.05$) increased by supplementation of probiotics in broiler diets compared to the control group. SIgM concentrations are indicators to reflect the humoral immunity situation in broiler chickens. Also, the excess occurred in the concentration of SIgM may refer to the addition of probiotics in broiler diets. The improvement in immunity of treated broiler chickens could be related to the inhibitory effects of probiotics on gut pathogens. Research shows that probiotics stimulate the immune system in many ways. Havenaar and Spanhaak [24] has reported that probiotics stimulate the immunity of the chickens in two ways (a) flora from probiotic migrate throughout the gut wall and multiply to a limited extent or (b) antigen released by the dead organisms are absorbed and thus stimulate the immune system. At present it is believed that there is some relationship between the ability of strain to translocate and the ability to be immunogenic. The improvement in the immune system may be refer to three different ways: (a) enhanced macrophage activity and enhanced ability to phagocytose microorganism or carbon particles; (b) increased production of antibodies usually of IgG and IgM classes and interferon (a nonspecific antiviral agent) and; (c) increased local antibodies at mucosal surfaces such as the gut wall [1].

Although it is well known that probiotic organisms may stimulate or interact with the host immune system, a general understanding of this relationship is complicated by our limited knowledge of how the avian immune system is regulated in the gut. The gut is often referred to as the largest immune organ of the body as more lymphocytes reside in the gut than in any other tissues. This is a reflection of the size of the gut and the amount of surface area in contact with the external environment. The enterocytes of the intestinal epithelium provide a barrier both to prevent the passive loss of nutrients and to prevent the access of pathogens to the body proper. The same barrier limits, however, the immune system’s ability to detect potential pathogens in the lumen. To circumvent this, pathogenic bacteria expressing appropriate genes penetrate the gut through M cells. M cells are scattered throughout the intestinal tract and comprise approximately 1% of total intestinal epithelia. The M cells are above regions of the lamina propria enriched for lymphocytes, macrophages, heterophils and dendritic cells. M cells have phagocytic properties and sample antigens originating from the gut lumen and transport them to nearby immune cells beneath [10].

Several studies have described the use of probiotics to enhance specific aspects of intestinal and/or systemic immunity. Numerous studies targeted at the specific effects of various probiotic organisms on the immune system have been conducted in mammals. These studies have suggested many different potential mechanisms for interactions and outcomes. Probiotics may have the ability to directly influence the inflammatory response elicited by pathogens by down regulating specific signaling pathways [61]. There are several pathways proposed for activation of immune response by gut microflora or when cells are infected by a variety of pathogens including those utilizing MAP kinase and NF-kappa beta pathways [41].

Probiotics modulate the expression of various pro- and anti-inflammatory cytokines. The results of these various studies are similar to those investigating the physiologic effects of probiotics in that biologic outcomes can vary greatly between strains of bacteria and even between species or genotype of the host. While the reports of probiotic effects on the immune response in chickens are more limited compared with mammals, similar results have been described. It has to be mentioned that the majority of these studies have examined effects on systemic immunity [10]. Yurong et al, [61] have described that the use of probiotics increased the amount of IgA found in the lumen, the numbers of IgA, IgM and IgG producing cells as well as the numbers of T cells in the cecal tonsils. These increases in immune parameters were accompanied by increased density of the microvilli and length of the cecal tonsils. Haghighi et al., [23] reported that oral administration of probiotic organisms increases natural antibodies (Abs) against several different antigens (Ag) in both the gut and the serum. Similarly, Zulkifli et al, [65] described an increased Ag-specific Ab response following probiotic treatment and Newcastle disease vaccination. The potential effects of probiotics on systemic immunity is not limited to just Ab responses. Farnell et al, [19] noted that the treatment of chickens with probiotics leads to a significant increase in the oxidative burst and degranulation of heterophils as compared to controls.

**Effect of probiotics in broiler diets on duodenum histomorphological examination:**

Effects of probiotics on circular muscular layer, muscular mucosa layer, villi height, villi width and villi space from duodenum of broiler chickens at 7 weeks of age are given in Table (7). Results of circular muscular layer and muscular mucosa layer
show that control group was recorded significantly \((P \leq 0.05)\) thick layers compared to other treated groups \((\text{Fig. 1})\). Supplementation of probiotics to broiler diets for a period of six weeks resulted in significantly \((P \leq 0.05)\) thin layers in both of circular muscular and muscular mucosa layers compared to control group \((\text{Fig. 1})\). These results mean that the thin layers of circular muscular and muscular mucosa may lead to be easier to transport of feed elements (nutrients absorption) from intestinal lumen to bloodstream than thick layers which appeared in the heavier body weights of treated groups than that of the control group.

The achieved results of villi height and villi width displayed that all groups of broiler chickens supplemented with probiotics were significantly \((P \leq 0.05)\) increased in villi height than the control group. Whereas group T4 showed significant \((P \leq 0.05)\) increase in villi width than all other groups including control one \((\text{Fig. 2})\). In contrary, group T4 recorded significantly \((P \leq 0.05)\) lower villi space compared to all other groups \((\text{Fig. 2})\). These results may reflect that probiotics improved the rate of metabolism for the treated groups more than untreated one via the increase the villi height. This increment in villi height may cause increase nutrients absorption due to increase of intestinal surface area for absorption \([46]\). The obtained results of histomorphological examination of broiler duodenum could be the reason that body weights and feed conversion of the treated groups were numerically superior than that of control group. The histomorphological changes in the intestine of broiler chickens reported in the present study provide new information regarding the potential for using probiotics in broiler diets. Increasing the villi height suggests an increased surface area capable of greater absorption of available nutrients \([8,46]\). The villi crypt is considered as the villi factory and deeper crypts indicate fast tissue turnover to permit renewal of the intestine within 48 to 96 h \([26,45]\). A shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance \([56]\). In contrast, increases in the villi height and villi height:crypt depth ratio are directly correlated with increased epithelial cell turnover \([18]\), and longer villi are associated with activated cell mitosis \([47]\). In the present study, supplementation of broilers with probiotic increased the villi height and villi width in broiler duodenum significantly \((P < 0.05)\), suggesting an increased epithelial cell turnover due to feeding of direct-fed microbes. Furthermore, it was shown that the addition of \textit{E. Faecium} to broiler diet increased the ileal villi height and enhanced broiler performance with respect to weight gain and FCR \([48]\) and addition of a probiotic containing lactobacilli, \textit{B. thermophilum}, and \textit{E. faecium} to the broiler diet increased the jejunal villi height \([11]\).

**Effect of probiotics in broiler diets on intestinal microbial examination:**

The effects of probiotics supplementation in broiler chicken diets on microbial organisms \((\text{population})\) from intestine of broiler chickens at 7 weeks of age are shown in Table \((8)\). The obtained results of microbial organisms from broiler chickens intestine at 7 weeks of age show big differences in the presence and absence of microorganisms as percentage in the chickens intestine during the examination. There was a clear reduction in the treated groups in \textit{E. Coli} compared to control group. \textit{E. Coli} was reduced from 27% of control group up to 5% of group T4. Control group recorded a highest content in \textit{E. Coli} \((27\%)\), followed by T2 was \((18\%)\) and then T3 \((10\%)\) and T4 was lowest in \textit{E. Coli} content \((5\%)\). Both of Proteus and Shegella contents were \(18\%\) in control group compared with \(9\%\) for both of Proteus and Shegella in group T2. While groups T3 and T4 registered \(0\%\) of both Proteus and Shegella organisms. These results demonstrate that probiotics of T3 and T4 were more effect to kill, eliminate or exclusion these types of microorganisms from the broiler chickens intestine during this study. Furthermore, both of \textit{Klebsella} and \textit{Enterobacter} microorganism were found at percent of 18 and 9% respectively for control group and were absent or missing in the intestine of broiler chickens of the other experimental groups. Also, \textit{Salmonella} and \textit{Edwardsell} bacteria were missed in all experimental groups including control one. On the other hand, supplementation of different types of probiotics in broiler chicken diets had a positive effect in improving the microflora contents in broiler intestine as seen in Table \((8)\). The microflora increased from 10% of control group to 64 %, 90% and 95% for groups T2, T3 and T4 respectively. These results showed how much the effect of these probiotics on protection of the broiler chickens from harmful effects of the pathogens and how much increased the useful microflora in broiler intestine. Use of such probiotics has helped to reduce the nonbeneficial bacteria. These results may attribute to that probiotics have a beneficial affect the host animal by improving its intestinal bacterial balance. Probiotic intake should result in the creation of gut microecological conditions that suppress harmful microorganisms and favor beneficial microorganisms and ultimately enhance gut health by immunomodulation and facilitating the elimination of pathogens. Probiotics and direct-fed microbial feed supplements have been confirmed in numerous scientific investigations to modulate the composition of the gut microflora by successfully competing with
pathogens through a competitive exclusion process [43]. Also, Probiotics modify the intestinal environment by reducing the pH, supplying digestion enzymes and increasing enzyme activity in the gastrointestinal tract [14,30]. Furthermore, probiotics induce alteration in intestinal flora, enhance the growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, and suppress the growth of intestinal pathogens [58]. On the other hand, the beneficial effects of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity. The suppression of bacterial numbers could be produced by production of antibacterial substances. Primary metabolites, such as organic acids and hydrogen peroxide, are known to be effective in vitro. However, the evidence for the involvement of organic acids in the control of gut bacteria is described as being produced by lactic acid bacteria, but some authors think that the observed inhibitory effects can, in many cases, be accounted for by low pH and primary metabolites. Another mechanism for preventing colonization by pathogens is competition for adhesion sites on the gut epithelial surface [3,13].

Based on the obtained results, it can be concluded that supplementation of probiotics to broiler chick diets led to increase in the percentage of muscular mucosal layers. Also, addition of probiotics and changes occurred in the circular muscular and mucosal layers. Also, addition of probiotics to broiler diets led to numerical increase in the percentage of useful microflora and reduced the pathogenic bacteria. Ultimately, addition of probiotics is recommended for broiler diets at the levels that mentioned in this study without any adverse effects.

### Table 2: Effects of probiotics in broiler chicken diets on body weight (g) during the period from 2 to 7 weeks of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Age</th>
<th>(1WK)</th>
<th>(2WK)</th>
<th>(3WK)</th>
<th>(4WK)</th>
<th>(5WK)</th>
<th>(6WK)</th>
<th>(7WK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>80.41 ±0.90</td>
<td>200.98 ±2.88</td>
<td>420.69 ±9.76</td>
<td>818.03 ±15.79</td>
<td>1322.99 ±24.14</td>
<td>1898.08 ±34.57</td>
<td>2457.00 ±44.91</td>
</tr>
<tr>
<td>T2</td>
<td>3.0ml/kg feed</td>
<td>80.33 ±1.16</td>
<td>204.28 ±4.58</td>
<td>444.38 ±9.53</td>
<td>835.46 ±15.27</td>
<td>1349.29 ±21.10</td>
<td>1927.79 ±29.00</td>
<td>2524.58 ±39.76</td>
</tr>
<tr>
<td>T3</td>
<td>0.5g/kg feed</td>
<td>80.48 ±1.24</td>
<td>203.94 ±3.55</td>
<td>443.95 ±7.73</td>
<td>800.91 ±13.21</td>
<td>1319.69 ±18.71</td>
<td>1912.58 ±27.49</td>
<td>2541.42 ±33.90</td>
</tr>
<tr>
<td>T4</td>
<td>1.0g/kg feed</td>
<td>80.38 ±1.11</td>
<td>210.66 ±3.33</td>
<td>458.95 ±7.51</td>
<td>823.88 ±14.04</td>
<td>1376.02 ±20.04</td>
<td>1970.83 ±25.96</td>
<td>2553.75 ±35.43</td>
</tr>
</tbody>
</table>

*Least squares means ± pooled standard error.

### Table 3: Effects of probiotics in broiler chicken diets on total feed intake (g/bird) during the period from 2 to 7 weeks of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Age</th>
<th>(2WK)</th>
<th>(3WK)</th>
<th>(4WK)</th>
<th>(5WK)</th>
<th>(6WK)</th>
<th>(7WK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td></td>
<td>221.83 ±11.69</td>
<td>642.50 ±10.58</td>
<td>896.50 ±10.71</td>
<td>1135.08 ±18.77</td>
<td>1185.75 ±16.19</td>
<td>1291.33 ±16.66</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>235.50 ±8.32</td>
<td>662.16 ±8.80</td>
<td>916.53 ±14.80</td>
<td>1076.29 ±16.79</td>
<td>1273.99 ±39.77</td>
<td>1232.80 ±22.80</td>
</tr>
<tr>
<td>T2</td>
<td>3.0ml/kg feed</td>
<td>232.50 ±5.11</td>
<td>650.50 ±6.61</td>
<td>865.50 ±4.16</td>
<td>1136.03 ±5.76</td>
<td>1208.80 ±2.75</td>
<td>1300.00 ±0.00</td>
</tr>
<tr>
<td>T3</td>
<td>0.5g/kg feed</td>
<td>277.50 ±12.05</td>
<td>636.75 ±4.25</td>
<td>878.57 ±3.02</td>
<td>1152.35 ±15.33</td>
<td>1185.75 ±12.75</td>
<td>1297.00 ±3.00</td>
</tr>
</tbody>
</table>

*Least squares means ± pooled standard error.

### Table 4: Effects of probiotics in broiler chicken diets on feed conversion (g feed/1g weight gain. bird) during the period from 2 to 7 weeks of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed conversion week 2</th>
<th>Feed conversion week 3</th>
<th>Feed conversion week 4</th>
<th>Feed conversion week 5</th>
<th>Feed conversion week 6</th>
<th>Feed conversion week 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.10 ±0.06</td>
<td>2.06 ±0.00</td>
<td>2.15 ±0.02</td>
<td>2.19 ±0.04</td>
<td>2.18 ±0.01</td>
<td>2.19 ±0.02</td>
</tr>
<tr>
<td>Control</td>
<td>1.15 ±0.04</td>
<td>2.03 ±0.04</td>
<td>2.13 ±0.04</td>
<td>2.16 ±0.04</td>
<td>2.17 ±0.02</td>
<td>2.18 ±0.01</td>
</tr>
<tr>
<td>T2</td>
<td>1.14 ±0.03</td>
<td>1.98 ±0.02</td>
<td>2.14 ±0.02</td>
<td>2.19 ±0.01</td>
<td>2.18 ±0.02</td>
<td>2.19 ±0.02</td>
</tr>
<tr>
<td>3.0ml/kg feed</td>
<td>1.15 ±0.04</td>
<td>2.03 ±0.04</td>
<td>2.15 ±0.04</td>
<td>2.17 ±0.02</td>
<td>2.18 ±0.01</td>
<td>2.19 ±0.02</td>
</tr>
<tr>
<td>T3</td>
<td>1.31 ±0.02</td>
<td>1.99 ±0.01</td>
<td>2.09 ±0.01</td>
<td>2.14 ±0.01</td>
<td>2.18 ±0.02</td>
<td>2.13 ±0.01</td>
</tr>
<tr>
<td>0.5g/kg feed</td>
<td>1.31 ±0.07</td>
<td>2.02 ±0.02</td>
<td>2.12 ±0.02</td>
<td>2.17 ±0.01</td>
<td>2.18 ±0.02</td>
<td>2.14 ±0.01</td>
</tr>
<tr>
<td>T4</td>
<td>1.0g/kg feed</td>
<td>2.07 ±0.01</td>
<td>2.14 ±0.01</td>
<td>2.18 ±0.02</td>
<td>2.20 ±0.02</td>
<td>2.21 ±0.02</td>
</tr>
</tbody>
</table>

*Least squares means ± pooled standard error.

### Notes:

1. Means having different letter exponents among columns are significantly different (P≤0.05).
Fig. 1: Shows the differences in the thickness of circular muscular layer (black arrows) and muscular mucosal layer (white arrows) from duodenum of broiler chicks at 7 weeks of age fed different types of probiotics.

Fig. 2: Shows the effect of probiotics supplementation on the differences in villi size (Width and height).

Table 5: Effects of probiotics in broiler chicken diets on absolute immune organ weights (g) at 7 weeks of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spleen weight (g)</th>
<th>Bursa weight (g)</th>
<th>Thymus weight (g)</th>
<th>Cecal tonsil weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>4.60±0.50</td>
<td>4.37±0.41</td>
<td>16.18±1.58</td>
<td>0.96±0.12</td>
</tr>
<tr>
<td>T2 3.0ml/kg feed</td>
<td>2.82±0.26</td>
<td>4.61±0.67</td>
<td>12.11±2.04</td>
<td>0.98±0.05</td>
</tr>
<tr>
<td>T3 0.5g/kg feed</td>
<td>3.12±0.42</td>
<td>2.73±0.55</td>
<td>16.16±1.76</td>
<td>0.99±0.10</td>
</tr>
<tr>
<td>T4 1.0g/kg feed</td>
<td>3.69±0.18</td>
<td>3.25±0.41</td>
<td>16.29±1.88</td>
<td>0.94±0.087</td>
</tr>
</tbody>
</table>

Least squares means ± pooled standard error.

a-b Means having different letter exponents among columns are significantly different (P≤0.05).
Table 6: Effects of probiotics in broiler chicken diets on serum total protein (g/l), albumin (g/l), globulin (g/l) and IgM (mg/dl) at 5, 6 and 7 weeks of age.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>5 weeks</th>
<th>6 weeks</th>
<th>7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>3.0ml/kg feed</td>
<td>3.20 ± 0.04</td>
<td>4.23 ± 0.47</td>
</tr>
<tr>
<td>T2 3.0ml/kg feed</td>
<td>3.43 ± 0.07</td>
<td>4.42 ± 1.14</td>
<td>7.92 ± 0.09</td>
</tr>
<tr>
<td>T3 0.5g/kg feed</td>
<td>3.30 ± 0.04</td>
<td>4.81 ± 0.06</td>
<td>7.92 ± 0.08</td>
</tr>
<tr>
<td>T4 0.5g/kg feed</td>
<td>3.53 ± 0.04</td>
<td>4.73 ± 0.05</td>
<td>7.73 ± 0.01</td>
</tr>
</tbody>
</table>

Least squares means ± pooled standard error.

Table 7: Effects of probiotics in broiler chicken diets on circular muscular layer (µm), muscular mucosa layer (µm), villi height(µm), villi width(µm) and villi space (µm) at 7 weeks of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Circular muscular layer</th>
<th>Muscular mucosa layer</th>
<th>Villi height</th>
<th>Villi width</th>
<th>Villi space</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>0.196 (a)</td>
<td>0.227 (a)</td>
<td>0.607 (a)</td>
<td>0.085 (a)</td>
<td>0.031 (a)</td>
</tr>
<tr>
<td>T2 3.0ml/kg feed</td>
<td>0.165 (a)</td>
<td>0.181 (a)</td>
<td>0.830 (a)</td>
<td>0.028 (a)</td>
<td>0.004 (a)</td>
</tr>
<tr>
<td>T3 0.5g/kg feed</td>
<td>0.189 (b)</td>
<td>0.198 (b)</td>
<td>0.759 (b)</td>
<td>0.033 (b)</td>
<td>0.005 (b)</td>
</tr>
<tr>
<td>T4 1.0g/kg feed</td>
<td>0.171 (c)</td>
<td>0.195 (c)</td>
<td>0.740 (c)</td>
<td>0.027 (c)</td>
<td>0.006 (c)</td>
</tr>
</tbody>
</table>

Least squares means ± pooled standard error.

Table 8: Effects of probiotics supplementation in broiler diets on microbial contents in intestine at 7 weeks of age.

<table>
<thead>
<tr>
<th>Microbial Organisms</th>
<th>(T1) Control</th>
<th>(T2) 3.0ml/kg feed</th>
<th>(T3) 0.5g/kg feed</th>
<th>(T4) 1.0g/kg feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>27%</td>
<td>18%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Proteus</td>
<td>18%</td>
<td>9%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shigella</td>
<td>18%</td>
<td>9%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsella</td>
<td>18%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>9%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Edwardsell</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Microflora</td>
<td>10</td>
<td>64</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

REFERENCES

Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. Poult. Sci., 86: 1121-1132.


