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Histological Response of Broiler's Immune Related Organs to Feeding Different Direct Fed Microbials

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Abstract: An experiment was conducted to study the effect of feeding different commercial preparations of direct-fed microbials (DFM) used as growth promoters on weight and histology of immune related organs (bursa, thymus and spleen) in broilers. Two hundred unsexed 10 days old Cobb broiler chicks were individually weighed and divided into 4 groups (5 replicates of 10 chicks, each) and fed 4 different experimental diets. A mixture of *Enterococcus faecium* (Protexin®, DFM1), a mixture of *Bacillus subtilis* (Clostat®, DFM2) and *Saccharomyces cerevisiae* yeast cells with its fermentation metabolites (Diamond®, DFM3) were supplemented to broiler diets and compared with the basal diet which served as control. Bursa, thymus and spleen were taken from birds at 36 days of age. The results showed significant ($P<0.01$) increase in spleen and bursa weight (relative to live body weight) in birds fed DFM supplemented diets compared with those fed the control diet of no supplement. Addition of DFM enhanced the activity of bursal follicles and may improve the bursa activity and caused improvement in thymus and spleen structure compared with the control. In conclusion, DFM supplemental levels have stimulated some histological change in the immune related organs which may result in improvement of chick immunity.

Key words: Probiotics, feed additives, broiler, histology, immune organs

INTRODUCTION

Recently direct fed microbials (DFM), probiotics, were introduced to use as growth promoters in broiler feeds to substitute sub-therapeutic antibiotics growth promoters which have been strictly banned in some areas of the world.

A probiotic is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance. It has been used as a substitute of antibiotics that is being used in considerable amounts as growth promoters in broilers production (Irshad, 2006).

Martins *et al*. (2005) stated that the mode of action of probiotics in the chickens is mainly due to low redox potential, reductions in the population of *Escherichia coli* competition for adhesion receptors in the intestine and reduction of toxin release. Probiotics may positively affect various physiologic functions in ways that will permit them now or in the future to be classified as functional foods for which health claims of enhanced production or reduction in disease risk (Irshad, 2006). Yakhkeshi *et al*. (2011) added that probiotics not only are used as a growth promoter that improve feed efficiency, but also induce immune system and have protective effects against many diseases.

Boushra *et al*. (2011) reported that addition of probiotics lead to hyperplasia of lymphoid follicles in bursa, thymus and spleen of broilers.

Inconsistent results had been published concerning the effect of feeding broilers on diets supplemented with DFM (probiotics) on immune related organs. Some authors (Willis *et al*., 2007, Alkhalf *et al*., 2010, El-Sheikh *et al*., 2009 and Tollba, 2010) reported that feeding broilers on diets supplemented with probiotics showed significant increase in bursa and spleen relative weights. Others (Awad *et al*., 2009 and Zhang *et al*., 2012) found that relative weights of bursa was unaffected by dietary inclusion of probiotic. However, the relative weight of spleen was increased by 3.8%.

Therefore, the objective of this work was to further evaluate the effects of feeding diets supplemented with different direct fed microbials on weights and histological characteristics of immune related organs (bursa, thymus and spleen) of broiler chicks.

MATERIALS AND METHODS

A total number of 200 unsexed 10 days old Cobb broiler chicks were individually weighed and divided among 4 groups (5 replicates of 10 chicks, each). The average initial live body weight of all replicates was nearly similar. Replicates were randomly allocated in batteries. The experiment involved examined three commercial products of different commercial preparations of direct-fed microbials (DFM) being Protexin® (a mixture of *Enterococcus faecium*, DFM1) produced by Novartis limited, international, UK, Clostat® (a mixture of *Bacillus subtilis*, DFM2) produced by Kemin Industries, Inc., USA.

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and Diamond® (*Saccharomyces cerevisiae* yeast cells with its fermentation metabolites, DFM3) produced by Diamond V Mills, Inc.

Birds were fed a grower diet contained 21% CP and 3150 Kcal ME/Kg from 11 to 25 days of age then switched to finisher diet contained 20% CP and 3200 Kcal ME/Kg from 26 to 36 days of age. Diets were formulated to cover all the nutrient requirements of Cobb broilers (Hassan *et al*., 2014). The formulation and nutrient composition of these diets are shown in Table 1. Diets were fed with no additives or supplemented with 0.01% Protexin® (DFM1), 0.06% Clostat ®(DFM2) or 0.05% Diamond® (DFM3). Supplementation levels were applied according to the producer recommendations. Birds were fed the experimental diets for *ad libitum* consumption. Birds were subjected to a vaccination program against AI, ND, IB and IBD throughout the experimental period.

Table 1: Formulation and nutrient composition of the basal grower and finisher diets

Ingredients %	Grower diet (11-25 d)	Finisher diet (26-36 d)
Yellow corn	61.47	62.2
Soybean meal (48%)	29	27.5
Corn gluten meal (60%)	2.5	2
Vegetable oil	3	4.5
Dicalcium phosphate	1.5	1.4
Limestone	1.25	1.3
Vitamin and mineral mix[1]	0.3	0.3
Salt	0.2	0.25
L-lysine HCl	0.25	0.15
DL-methionine	0.2	0.13
Choline HCl	0.13	0.1
Threonine	0.1	0.07
Phytase	0.1	0.1
Total	100	100
Calculated Composition[2]%		
Crude protein	21.1	20
ME (Kcal/Kg)	3142	3237
Lysine	1.31	1.18
Methionine	0.55	0.48
Meth + Cystine	0.92	0.81
Threonine	0.85	0.75
Calcium	0.9	0.86
Nonphytate P	0.4	0.37

[1]Vitamin-mineral mixture supplied per kg of diet: Vit. A, 12,000 IU, Vit. D3, 2,200 IU, Vit. E, 10 mg, Vit. K3, 2 mg, Vit. B1, 1 mg, Vit. B2, 4 mg, Vit. B6, 1.5 mg, Vit. B12, 10 ìg, Niacin, 20 mg, Pantothenic acid, 10 mg, Folic acid, 1 mg, Biotin, 50µg, Copper, 10 mg, Iodine, 1 mg, Iron, 30 mg, Manganese, 55 mg, Zinc, 50 mg and Selenium, 0.1 mg.

[2]Calculated values based on feed composition Tables of NRC (1994).

At the end of the experiment, 2 birds per replicate were slaughtered, eviscerated and bursa, spleen and thymus were removed. Weight of bursa and spleen were recorded as % of live body weight. Representative tissue samples from immune related organs (bursa, thymus, spleen) were taken to study the histological changes associated with the experimental treatments. Samples were fixed in a 10% formalin-saline solution before preparing the histological sections using paraffin method technique. All sections were dehydrated in ascending grades of ethanol, cleared in zylene and then embedded in paraffin wax. Transverse sections (4-5 microns, thickness) were taken, mounted on glass slides and stained with haematoxylin and eosin (H&E) stains. All sections were examined under electric microscope provided with computerized Camera.

Data of weights of bursa and spleen were statistically analyzed for analysis of variance using the General Liner Model of SAS Institute (1990). Significant differences among treatment means were separated by Duncan's multiple rang test (Duncan, 1955).

RESULTS

Relative weight of bursa and spleen: The effect of dietary treatments on the relative weights of bursa and spleen are shown in Table 2. The results showed that the relative weight of bursa and spleen significantly ($p<0.01$) improved by supplementation of probiotic compared to the basal diet. No significant differences were observed on relative weight of bursa and spleen among the different direct fed microbials used.

Table 2: Effect of dietary treatments on relative weight of bursa and spleen (% of live body weight) of broiler chicks at 36 days of age

Item	Bursa %	Spleen %
Dietary Treatments		
Control (*No additives*)	0.08[b]	0.09[b]
DFM1	0.12[a]	0.13[a]
DFM2	0.11[ab]	0.13[a]
DFM3	0.12[a]	0.13[a]
Statistics		
SE of means	±0.01	±0.03
Significances	**	**

DFM1: a mixture of *Enterococcus faecium*.

DFM2: a mixture of *Bacillus subtilis*.

DFM3: *Saccharomyces cerevisiae* yeast cells with fermentation metabolites.

a-d Mean within each column with no common superscript differ significantly. ** $p<0.01$

Histological observation

Bursa of Fabricius: The bursa of Fabricius is a primary lymphoid organ in birds, and it is composed of about 15 plicae (folds), each contain numerous bursal follicles (F). These follicles have two distinct areas, cortex and medulla (Hodges, 1974). The present sections show that the F of the control group [Fig. 1(a)] are elongated with few large lymphocytes (LL) and abundant medullary area. This area is composed of apparently undifferentiated epithelial cells (pale-staining) and small lymphocytes (SL) or lymphoblasts. However, a well-developed bursal follicles could be seen in the DFM1

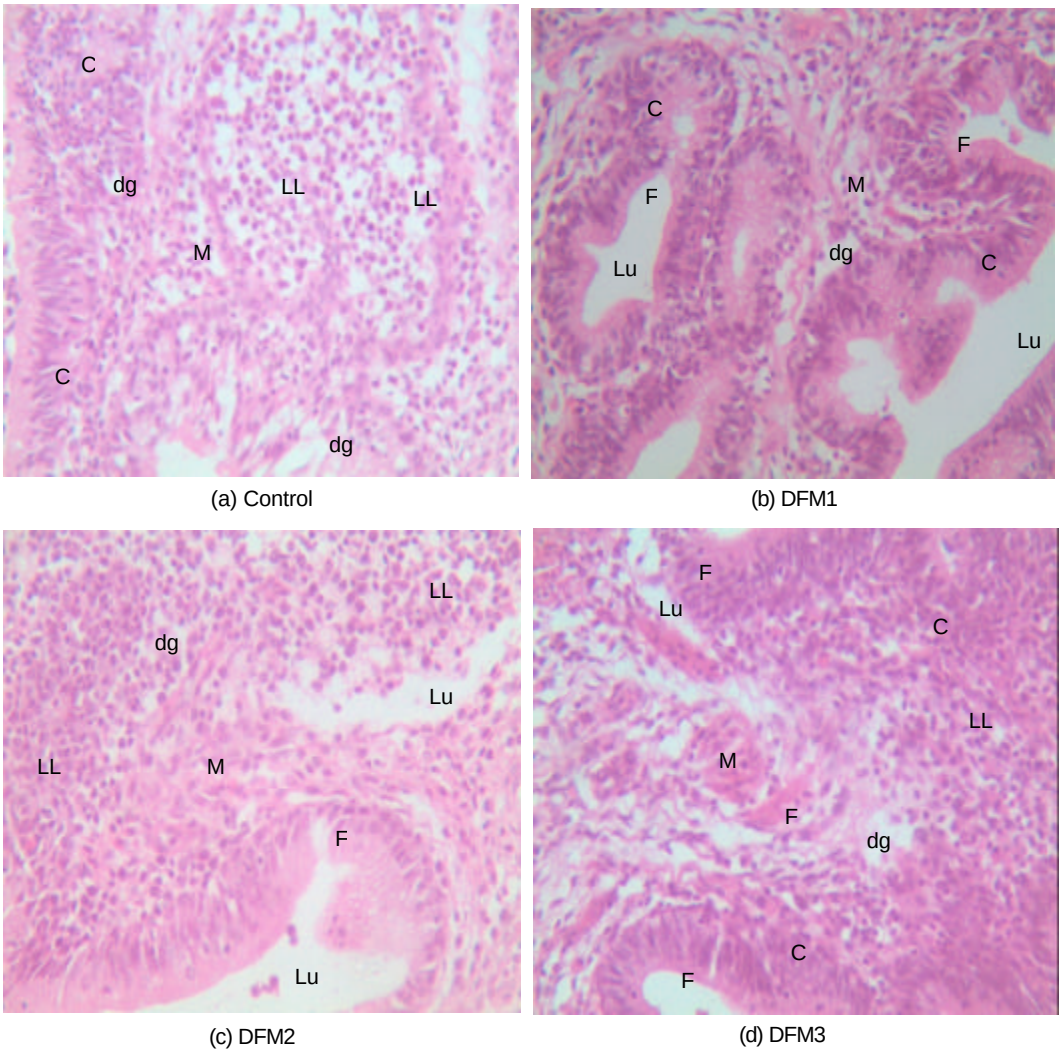

Fig. 1: Transverse section through bursa of Fabricius
F = bursa follicles, LL = large lymphocytes, Lu = lumen, C = cortex, M = medulla, SL = small lymphocytes, dg = degenerative area

and DFM2 section (Fig. 1b and c) and DFM3 section (Fig. 1d). It is clear from these sections that the DFM3 addition enhances the activity of bursal follicles. This hyperactivity is accompanied by the presence of many large lymphocytes in the cortex area concomitant with many lumens between the follicles. These lumens are abundant in all treatments especially in Fig.1 (b and c) and to less extent in Fig. 1d (DFM3). In general, the lumens are responsible for the phagocytic processes and for maintaining the B- cell production. It is also same age- related degenerative areas (dg) that could be seen with high frequency in Fig. 1a. From the previous observation, it appears that the direct- fed microbials (probiotics) may improve the bursa activity in treated birds and improved the immunity which is supported by results of lymphoid organ weight.

Thymus: The histological structures of thymus gland in response to different treatments are illustrated in Fig. 2. In general, the gland is enclosed by a fine connective tissue capsule with numerous fine septa which divide the gland into lobules (Khenenou *et al*., 2012). In the present sections, there is one lobule in each section with or without the fine septa in some sections (Fig.2a and b). Moreover, the distinction between the cortex and medulla had not been obvious in most sections. It is likely that the changes in thymus structure are related to dietary supplements of DFM. An increased number of large lymphocytes (LL) could be seen in Fig. 2 (b, c and d) and small lymphocytes in Fig. 2a (control). However, there were several types of cellular degeneration and vacuolation including necrotic areas concomitant with an irregular arrangement of thymic cells within cortex and

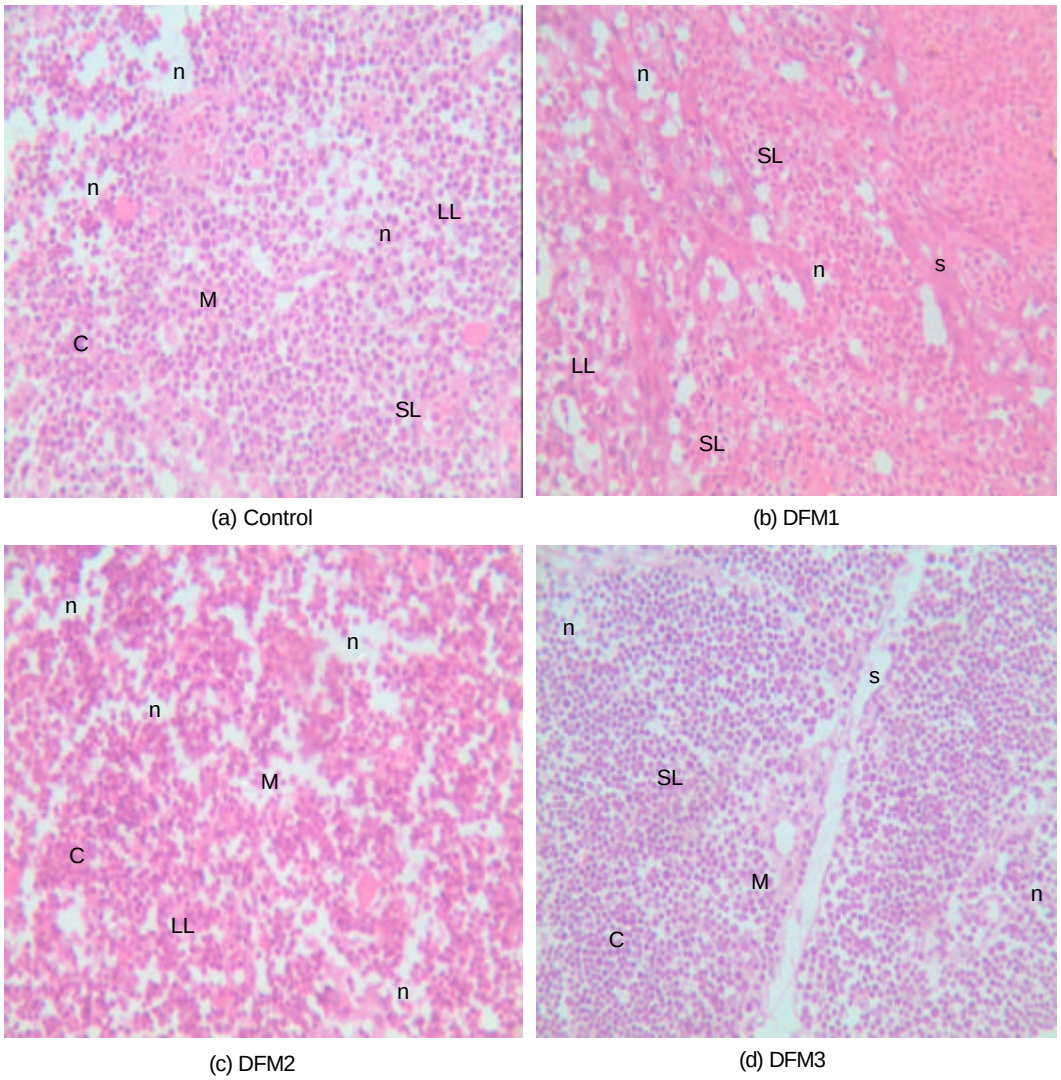

Fig. 2: Transverse section through the thymus gland
C = cortex, M = medulla, s = fine connective tissue septa, L, SL = large small lymphocytes, n = necrotic area

medullary region since, the gland undergoes involution with age, the changes may be in lymphocytes number and size along with the presence of degenerative or necrotic areas these changes are more evident in the control (Fig. 2a).

Spleen: The histological section of spleen from the control group (Fig. 3a) shows the basic structure of the splenic tissues, where a large white pulp (WP) area and a dark- stained red pulp (RP) area could be seen. Also, numerous blood vessels, sinusoids and lymphocytes of different size are observed. The main histological changes were detected in the DFM1 and DFM2 (Fig. 3b and c) treatment groups there were large numbers of small and large lymphocytes in both RP and WP areas. This was observed also in the DFM3 (Fig. 3d) treatment group but with the RP area extended all over the splenic tissues. In all sections, one can observe many basophilic hemosiderin granules between the blood sinusoids accompanied with different patterns of WP and RP distribution within each section.

DISCUSSION

The results of the present study indicated that broiler chicks fed probiotic in their diets had better immune response and disease resistance. In this respect, Katanbaf *et al.* (1989) reported that the increase in the relative weight of lymphoid organs is considered as an indication of the immunological advances. Heckert *et al.* (2002) proved that measurement of the immune organ weight is a common method of evaluating the immune status in chickens.

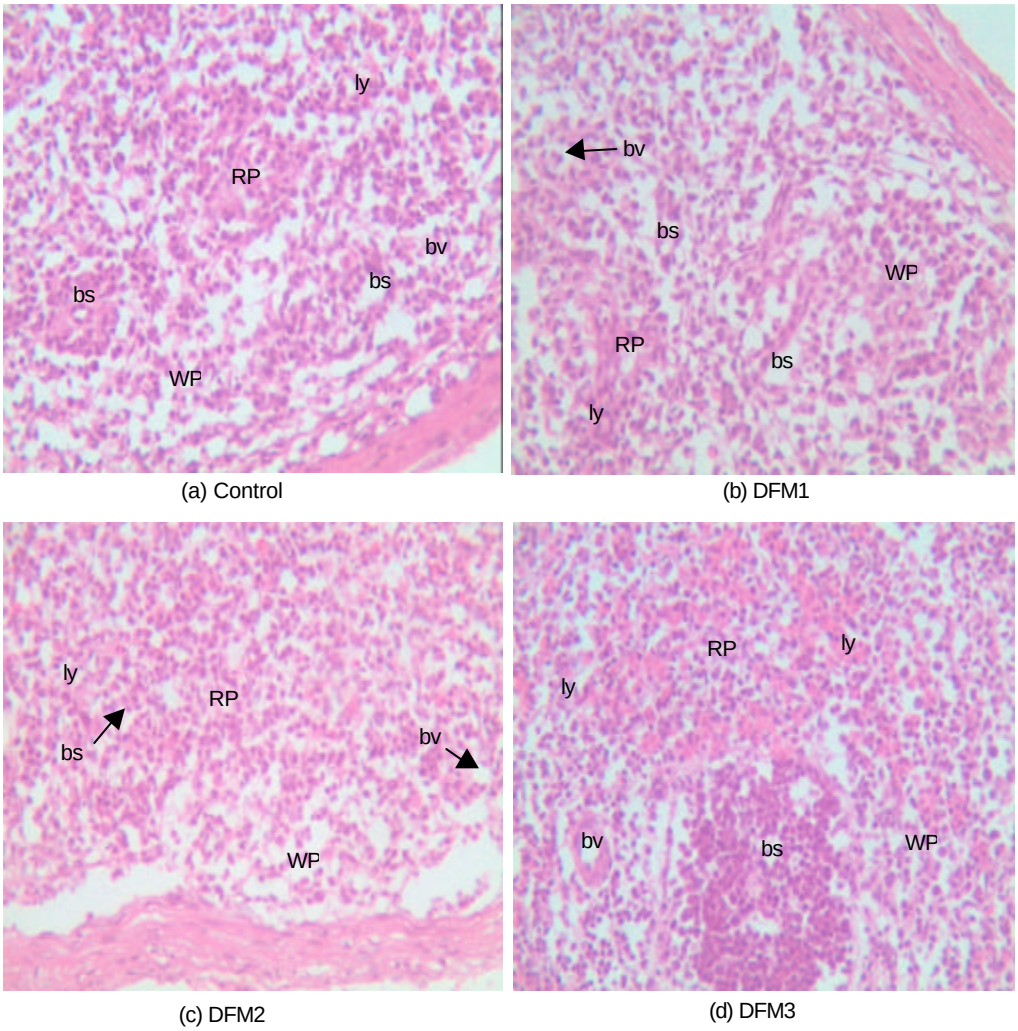

Fig. 3: Transverse section through the spleen.
RP= red pulp, WP= whit pulp, bv= blood vessel, bs= blood sinusoid, ly= lymphocytes

Teo and Tan (2007) observed that the birds provided feed supplemented with *Bacillius subtilis* PB6 had a significantly heavier bursa weights compared with the control group. However, neither the studied treatments nor the control had any effect on the relative weights of spleen. Also, Willis *et al*. (2007) found that the bursa and spleen relative weight significantly increased in male broiler chickens receiving the probiotic supplementation. The reported increase in weight bursa and spleen was confirmed by the histological observations of both organs.

It appears that the probiotics (DFM) have the ability to improve the immune responses of broiler chicks via different mechanisms. This was confirmed in previous results which showed that probiotics improve productive performance, modulate immune responses and promote protein synthesis and nutrient digestion and absorption (Xu *et al*., 2003; Pelicano *et al*., 2004 and Alkhalf *et al*., 2010). Therefore, the relative weights increase of bursa and spleen could be used as indicators of better immunity of broiler that fed probiotics. Similar results was also reported by Alkhalf *et al*. (2010), El-Sheikh *et al*. (2009) and Tollba (2010) who found significant increases in the relative weights of bursa and spleen as a consequence of feeding diets supplemented with direct- fed microbials.

The present observations support the findings of many workers who stated that the growth promoter addition sustains appropriate immune response in different avian and animal species (Mroz *et al*., 2006 and Hu and Guo, 2007).

The enhanced growth performance as seen in a previous report of the same authors (Hassan *et al*., 2014) is associated with significant increases in

lymphoid organs relative weights, indicative of better immune response and good physiological status of birds. These findings may explain and supported the result of Yakhkeshi *et al.* (2011) and Hassan *et al.*, (2014) who reported that important characteristics of probiotics are improving feed efficiency without any residual in the poultry tissue and resulting in diseases resistance. In addition, probiotics not only are used as a growth promoter, but also induce immune system and have protective effects against many diseases. Also, Alkhalf *et al.* (2010) found that a significant increase was recorded in the relative weight of bursa of Fabricius and spleen in all probiotic treatment groups at 42 days of age as compared to the control group.

On the other hand, Awad *et al.* (2009) and Zhang *et al.* (2013) found that relative weights of bursa of Fabricius was unaffected by dietary inclusion of $10^9 kg^{-1}$ *B. subtilis*. However, the relative weight of spleen was increased by 3.8% by adding *B. subtilis* in broiler diets.

Zhang *et al.* (2013) found that dietary supplementation with *B. subtilis* diets had 30.9% greater relative weight of thymus than those fed diets without probiotic while the relative weights of bursa of Fabricius and spleen were not affected.

Probiotics may stimulate different subsets of immune system cells (Bal *et al.*, 2004). Also, probiotics can significantly affect the systemic immune responses (Dallout *et al.*, 2003 and Landy and Kavyan, 2013). It is now well recognized that bioactive peptides released during fermentation by lactic acid bacteria could contribute to the known immunomodulatory effects of probiotic bacteria (Leblanc *et al.*, 2004). Also, probiotics can increase the systemic antibody production to some antigens in broilers (Haghighi *et al.*, 2005). Boushra *et al.* (2011) reported that addition of probiotics lead to hyperplasia of lymphoid follicles in bursa, thymus and spleen of broilers.

It is concluded that probiotics increased weight and stimulated some histological change in the immune organs, which improve bird's immunity.

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REFERENCES

Alkhalf, A., M. Alhaj and I. Al-Homidan, 2010. Influence of probiotic supplementation on immune response of broiler chicks. Egypt. Poult. Sci., 30: 271-280.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Bohm, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci., 88: 49-55.

Bal, A.P., Q. Ouyang, W. Zhang, C.H. Wang and S.F. Li, 2004. Probiotics inhibit TNF-a-induced interleukin-8-secreation of HT29 cells. World J. Gastroenterol., 10: 455-457.

Boushra, M.H., M.M. El-Hamamy, Amina A. Dessouki, and Rania T. Hassan, 2011. Pathological study on the effect of probiotic in chickens infected with *E. coli*. Suez Canal Vet. Med. J., XVI: 177-193.

Dallout, R.A., H.S. Lillehoj, T.A. Shellem and J.A. Doerr, 2003. Enhanced mucosal immunity against Eimeria acervulina in broilers fed a *Lactobacillus* based probiotic. Poult. Sci., 82: 62-66.

Duncans, D.B., 1955. Multiple range and multiple F test. Biomet., 11: 1-42.

El- Sheikh, T.M., E.A. Abdalla and M.M. Hanafy, 2009. Study on productive performance, hematological and immunological parameters in a local strain of chickens as affected by mannan- oligosaccharide under hot climate conditions. Egypt. Poult. Sci. J., 29: 287-305.

Haghighi, H.R., J. Gong, C.L. Gyles, M.A. Hayes, H. Zhou, B. Sanei, P. Parvizi, H. Gisavi, J.R. Chambers and S.H. Sharif, 2005. Modulation of antibody mediated immune response by probiotics. Clin. Diagn. Lab. Immunol., 12: 1387-1392.

Hassan, H.M.A., Amani W. Youssef, Eman F. El-Daly, Nafisa A. Abd El- Azeem, Eman R. Hassan and M.A. Mohamed, 2014. Performance, caecum bacterial count and ileum histology of broilers fed different direct-fed microbials. Asian J. Poult. Sci., 8: 106-114.

Heckert, R.A., I. Estevez, E. Russek-Cohen and R. Pettit-Riley, 2002. Effects of density and perch availability on the immune status of broilers. Poult. Sci., 81: 451-457.

Hodges, R.D., 1974. The histology of the fowl. 1st Ed.Academic Press, Inc (London) Ltd.

Hu, Z. and Y. Guo, 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Anim. Feed Sci. Tech., 132: 240-249.

Irshad, A., 2006. Effect of probiotics on broilers performance. Int. J. Poult. Sci., 5: 593-597.

Katanbaf, M.N., E.A. Dunnington and P.B. Siegel, 1989. Restricted feeding in early and late-feathering chickens. 1. Growth and physiological responses. Poult. Sci., 68: 344-351.

Khenenou, T., M. Mohamed, B. Omar and B. Hassina, 2012. Morpho histological study of the thymus of broiler chickens during post-hashing age. Int. J. Poult. Sci., 11: 78-80.

Landy, N. and A. Kavyani, 2013. Effects of using a multi-strain probiotic on performance, immune responses and cecal microflora composition in broiler chickens reared under cyclic heat stress condition. Iranian J. Appl. Anim. Sci., 3: 703-708.

Leblanc, J., I. Fliss and C. Matar, 2004. Induction of a humoral immune response following an *Escherichia coli* O157:H7 in-fection with an immunomodulatory peptidic fraction derived from *Lactobacillus* helveticus-fermented milk. Clin. Diagn. Lab. Immunol., 11: 1171-1181.

Martins, F.S., R.M. Nardi, R.M. Arantes, C.A. Rosa, M.J. Neves and J.R. Nicoli, 2005. Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. J. Gen. Appl. Microbiol., 51: 83-92.

Mroz, Z., S.J. Koopmans, A. Bannink, K. Partanen, W. Krasucki, M. Overland and S. Radcliffe, 2006. Carboxylic acids as bioregulators and gut growth promoters in nonruminants. In: Mosenthin, R., J. Zentek, T. ¯ebrowska (Editors). Biology of Nutrition in Growing Animals. Elsevier, Edinburgh (UK), pp: 81-133.

National Research Council - NRC, 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy press, Washington, DC.

Pelicano, E.R., P.A. Souza, A. Oba, K.A. Norkus, L.M. Kodawara and T.M. Lima, 2004. Performance of broilers fed diets containing natural growth promoters. Revista. Brasileira de Ciencia Avicola. 6: 231- 236.

SAS Institute, 1990. SAS/ STAT User's Guide: Statistics,® Version 6, 4th edition. SAS Institute Inc, Cary, NC.

Teo, A.Y. and H.M. Tan, 2007. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with bacillus subtilis PB6 (CloSTAT). J. Appl. Poult. 16: 296-303.

Tollba, A.A.H., 2010. Reduction of broilers intestinal pathogenic micro- flora under normal or stressed conditions. Egypt. Poult. Sci. J., 30: 249-270.

Willis, W.L., O.S. Isikhuemhen and S.A. Ibrahim, 2007. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult. Sci., 86: 1856-1860.

Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang, 2003. Effect of dietary fracto- oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broiler. J. Anim. Sci., 82: 1030-1036.

Yakhkeshi, S., S. Rahimi and N.K. Gharib, 2011.The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. J. Med. Plants, 10: 80-95.

Zhang, Z.F., J.H. Cho and I.H. Kim, 2013. Effects of Bacillus subtilis UBT-MO_2on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livest. Sci., 155: 343-347.

Zhang, Z.F., T.X. Zhou, X. Ao and I.H. Kim, 2012. Effects of beta-glucan and Bacillus subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize-soybean meal based diets. Livest. Sci., 150: 419- 424.