Histopathological changes of lymphoid system in broiler chicks after treatment with *lactobacillus acidophils* under heat stress

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Abstract

The study was conducted to investigate the effect of *lactobacillus acidophilus* on infected broiler chicks with *E.coli.* The study included 80 broiler chicks that are one day old which were divided into four equal groups (n=20). Group 1 one was given the lactobacillus acidophilus at one day old in dose of 1×10^8 CFU/0.1 orally while group 2 was given the same dose but at 21 day old. Group 3 was given the highly pathogenic *E.coli*at dose of 1×10^9CFU/ml at 35 day old and was regarded as the positive control. Group 4 was given the physiological saline solution and was regarded as the negative control. All three groups were exposed to heat stress, at the age of 31day old 50% of the total number were killed and the lymphoid tissue were extracted, these include: cecaltonsils, thymus gland and Harderain gland which were fixed in 10% neutral formalin. The remaining chicks were injected with *E.coli* in a dose of 1×10^9CFU/ml at the age of 35 days. All birds were scarified at the age of 38 days to investigate the lymphoid tissue and for their fixation in formalin. The result for the positive control showed that there were sloughing of the mucosal layer at the cecal tonsil in addition to heterophil infiltration of sub mucosa of Harderain gland and vacculation of epithelail cell lining of the Harderain gland, the thymus gland showed heavy lymphocytic infiltration in 2nd group while group 1 showed moderate hyperplasia of the lymphatic tissue. We concluded that *lactobacillus acidophilus* play an important role in the protection against *E.coli* infection under heat stress especially when they are given after the development of the immune system.

التغيرات المرضية الحاصلة في لوز الاعورين والتوثة وغدة هاردر بعد المعالجة بالعصيات اللبنية واجراء التحدي بالاشريشيا القولونية في فروج اللحم

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الخلاصة

اجريت الدراسة لمعرفة تأثير العصيات اللبنية على افراخ مصابة بجراثيم الاشريشيا القولونية .اشتملت الدراسة على 80 فرخة لحم عمر يوم واحد قسمت عشوائيا إلى اربعة مجاميع متساوية (n=20). أعطيت المجوعة الأولى العصيات اللبنية عند عمر يوم واحد وبجرعة قدرها 0.1/ 1×10^8CFU فمويا فيما أعطيت المجوعة الثانية نفس الجرعة ولكن عند عمر 21 يوم. أعطيت المجموعة الثالثة بعمر 35 يوم جراثيم الاشريشيا القولونية الضارية بجرعة 1×10^9CFU/ml وقد تم اعتبار هذه المجموعة على أنها مجموعة السيطرة الموجبة. تم إعطاء المجموعة الرابعة المحلول الفسيولوجي واعتبارها مجموعة السيطرة السالبة. عرضت المجاميع الثلاث إلى إجهاد حراري، عند عمر 31 يوم تم قتل 50% من العدد الكلي واستخراج الأعضاء اللمفاوية وهي: لوز الأعورين، غدة التوثة وغدة هاردر وتثبيتها بالفورمالين المتعادل بتركيز 10%، أما النصف الثاني فقد حقنت بجراثيم الاشريشيا القولونية 1×10^9CFU/ml وبعمر 35 يوم. تم قتل جميع الأفراخ عند عمر 38 يوم واستخراج الأعضاء اللمفاوية وتثبيتها بالفورمالين. أظهرت نتائج مجموعة السيطرة الموجبة انسلاخات لخلايا البطانة الطلائية عند منطقة اللوز في الاعورين بالاضافة الى ارتشاح الخلايا المتغايرة في الطبقة تحت الطلائية

المبطنة لغدة هارديرء كما أظهرت الغدة التوثة للمجموعة الثانية نفاد شديد للنسيج اللمفـــاوي، أمـــا المجموعـــة الأولى فقد أظهرت فرط تنسج طفيف للنسيج اللمفاوي. نستنتج من هذه الدراسة بان العصيات اللبنية لهـــا دور كبير في حماية الأفراخ عند الإصابة بالاشريشيا القولونية تحت تأثير الاجهاد الحراري خصوصا عند إعطاءهـــا في عمر قد أكتمل فيه الجهاز المناعي.

Introduction

The use of probiotic for control of pathogenic microorganism such as *E.coli* as an alternative to anti microbial growth promoters (AGP) and capability of modulating immune responses, has gained interest (1). Probiotic are defined as live microorganisms which, when administered in adequate amounts, confer ahealth benefit on the host through improvements to the intestinal microbial balance (2) specially when chicks get stress from anumber of factors such as overcrowding, unfavorable ambient medium, feed intake and vaccination (3). Lactic acid-producing bacteria (LAB) are normal residents of the chicken intestine, *Lactobacillus* is a genus of gram positive facultative anaerobic bacteria that make up the major component of the LAB group, which are the most common types of bacteria used as probiotics (4). The intestinal microbiota is inconstant contact with cells of the gut-associated lymphoid tissue (GALT), which includes professional antigen-presenting cells, B- cells, T-cells and intestinal epithelial cells, cecal tonsils are the major lymphoid tissues within the GALT (1), also the thymus gland evolved as primary lymphoid organ in chicken, that is generation of large and selected T-cell-repertoire (5, 6), Harderian gland is considered as secondary lymphoid organ play areole in local immunity (7). The declining use ofantibiotic for prevention and treatment of collibacilosis has stimulated interest in alternative methods including probiotics which are widely available for use in poultry (8). The evidence is reveal of no effect of feed additive IMBO® (containing *Enterococcus* & algae extracts) on histopathology of New castle disease virus in broiler chicken (9). However, information concerning the effect of *L.acidophilus* on histopathological changes of thymus, cecal tonsils and harderian gland in broiler under heat stress is not recorded. As such, the objective of the current study was to examination the effect probiotic (*L. acidophilus*) on hisopathological changes of these organs, under heat stress and challenge with *E.coli* to measure protective percent.

Materials and Methods

- **Chicken and Housing**: Newly hatched commercial broiler chicks of the Rose breed were used. Bird were maintained in floor pens on clean wood shavings at the field experimental Unit.Chicks were provided with free access to water and feed. The research was approved by the pathology and avian disease department/colloge of Veterinary Medicine/ Baghdad University.
- **Probiotic Isolates**: *Lactobacillus acidophilus* isolate was obtained from college of Agriculture; with concentration 1×10^8/0.1 CFU. and gavaged (1ml) to the crop.
- **Challenge Test and Preparation of bacterial Inoculation**: The bacterial strain used in this study as challenge stain was antigenically untyped *Escherichia Coli* that was supplied by Department of pathology and avian diseases/ College of Vet. Med. Baghdad University. The preparation of bacterial inoculation described by (10) was applied in which *E.coli* strain was inoculated into brain heart infusion broth and inoculated for 24 hr. at $37C^O$ then on MacConkey agar for 24 hr. at $37C^O$ after that it was counted according to Miles-Misra technique (11). Broiler chicks of 1st and 2nd groups were inoculated by gavages into the crop with 1×10^9cfu/ml. concentration of *E.coli* (12).

- **Hisopathological test**: Specimens were taken from the internal organs includes; cecal tonsils, thymus and harderian gland. The tissues were fixed in 10% buffer formaldehyde solution immediately after their removal. The processing was routinely done and then slides were stained with Hematoxilne and Eosine stain (13, 14). and histopathological changes were observed under light microscope.
- **Experimental design**: 80 chicks were randomly divided into four groups equally distributed (n=20) . The 1st group: given *L. acidophillus* in one day old; 2nd group: given *L.acidophilus* in 21 day old (3wk) and 3rd group: was regarded as control positive. All three groups were exposed to heat stress (38-40C^0) for 3hr/ day for 10 days, starting in 21 day old.
- **The fourth group**: was given normal saline (control negative).

In 31 day old half chicks from each group were sacrificed and lymphoid organs were taken. The challenge test was carried out at 35 day old with *E.coli* for the second half chicks from each group. All chicks were sacrificed and 3 day post infection period and the lymphoid organs (cecal tonsil, thymus and Harderian gland) were taken for histopathology study.

Results

- **Microscopic examination before challenge was characterized by**: cecal tonsils of 2nd group showed sever lymphocytic cell aggregation between mucosal glands where as in the 1st group there were few mononuclear cells infiltration between mucosal glands and lamina propria (Fig.1) compare with 3rd group which show no clear lesion (Fig.2).

In the 2nd group there were intensive lymphoid hyperplasia within the follicle of the thymus (Fig. 3) as compare to that reported in 1st group, the 1st group showed hyperplasia of lymphoid follicles in the sub capsular region (Fig.4). 3rd show normal structure of the thymus (Fig.5). In Harderian gland (Hg.) in the 2nd group showed marked aggregation of lymphocyte between glandular acini of the Hg. (Fig.6), the Hg. in the 1st group showed lymphocytic infiltration between glandular acini of hg. (Fig.7). In 3rd group there was no clear lesion (Fig.8).

- **Challenge test**: The results show as illustration in table (1).

Table (1) Mortality rate &Mortality percentage among experimental groups post inoculation with *E.coli*

Groups	No. of chicks group	Postinoculation with E.Coli mortality rate after 3 days	Mortality percentage%
G1	10	3/10	30%
G2	10	2/10	20%
G3	10	8/10	80%

- **Microscopic examination after challenge characterized by**: The 2nd group In cecal tonsil showed increase thickness of capsular region of lymphoid tissue due to proliferation of fibrous connective tissue. In addition other sections there was hyperplasia of goblet cells in the epithelial tissue with mononuclear cell infiltration in subepitheilal layer (Fig.9).

The main histopathological lesion in the cecal tonsil of 1st group showed moderate hyperplasia of lymphoid tissue and hyperplasia of the epithelial laying cell of the mucosal gland and fibrosis of the capsular wall were seen (Fig.10). The control group showed inflammatory cells particularly heterophilic infiltration in the epithelial layer and subepithelial layer with sloughing of the epithelial cell (Fig.11). Microscopic examination of thymus in the 2nd group showed marked hyperplasia of lymphoid tissue (Fig.12). While 1st group the thymus showed moderate hyperplasia of lymphoid tissue with moderate thickness in the capsular region (Fig.(13). Thymus of the positivecontrol revealed marked depletion of lymphoid tissue (Fig.14).

The Harderian gland in the 2nd group showed mononuclear cell infiltration in the subepithelial layer and with focal hyperplasia (Fig.15). The main lesion in 1st group characterized by vaculation of epithelial lining cell with mononuclear cell infiltration and focal hyperplasia of epithelial lining cell of the gland, In addition lymphoid tissue hyperplasia(Fig.16) .In 3rd reveal sever vacculation lesion post infection with *E.coli* (Fig.17).

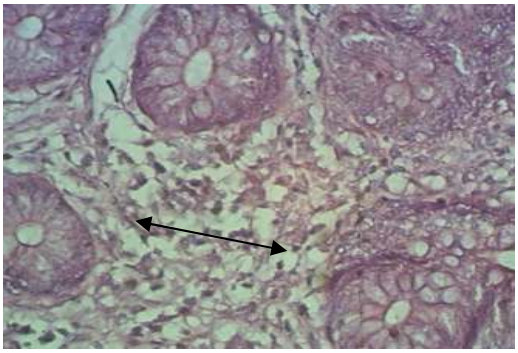

Fig. (1) Histological section in the cecal tonsil of 1st group shows few mononuclear cell infiltration between mucosal gland and lamina propria◄——► (H & E stain 40×)

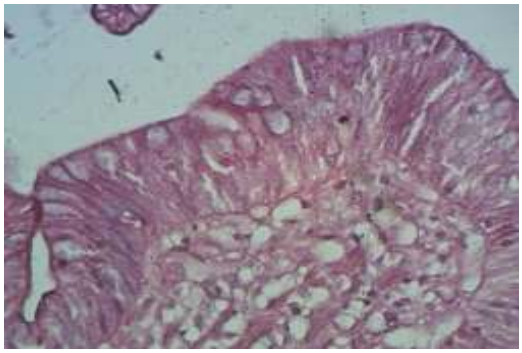

Fig.(2):Histological section in the cecal tonsil of control group shows no clear lesion (H&E stain 40X)

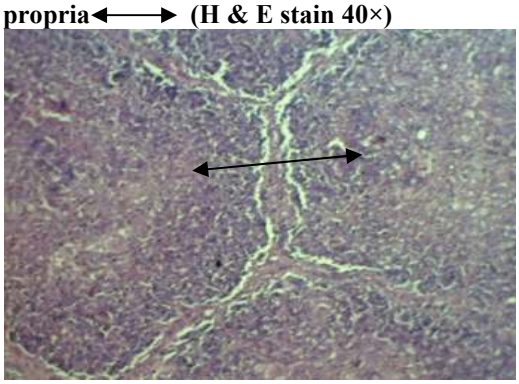

Fig. (3) Histological section in the thymus in 2nd shows marked hyperplasia in the lymphoid follicles of the thymus◄——► (H & E stain 40X)

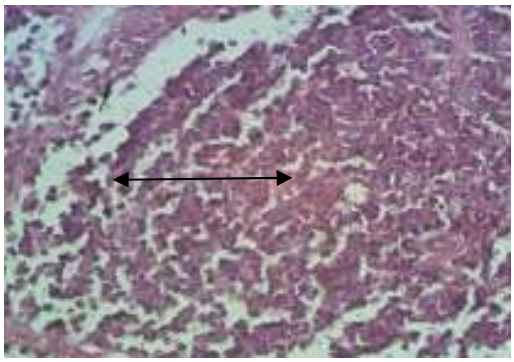

Fig. (4) Histological section in the thymus of 1st group shows moderate hyperplasia of lymphoid follicles and lymphocyte appear in the sub capsular region ◄——► (H &E stain 40X)

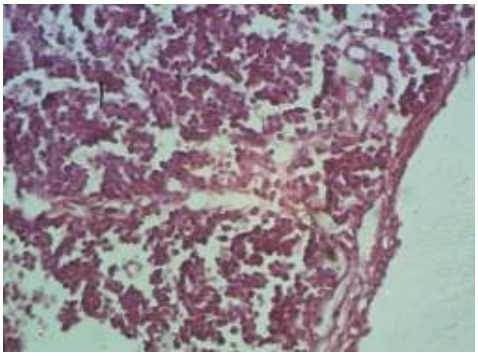

Fig. (5) Histological section in the thymus of control group shows normal structure of the thymus (H & E stain 40 X).

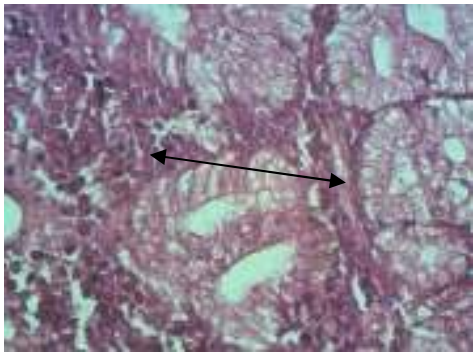

Fig. (6) Histological section in the Harderian gland of 2nd group shows marked lymphocyte aggregation between glandular acini of the Hg ◄——► (H & E stain 40 X)

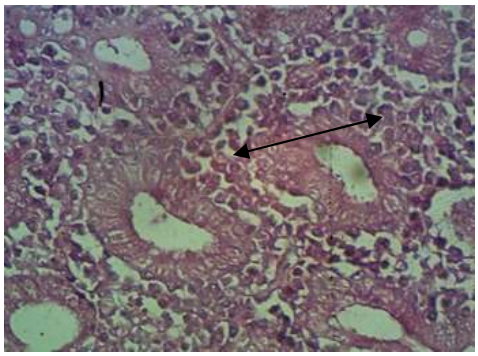

Fig. (7) Histological section in the Harderain gland of 1st group shows lymphocytic infiltration between acini Harderain gland ⟷ (H & E stain 40 X)

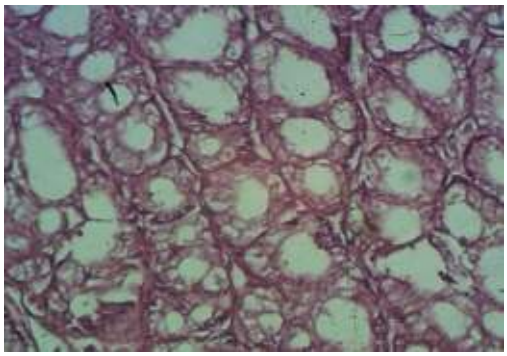

Fig. (8) Histological section in the Harderain gland of control group shows no clear lesion (H & E stain 40X)

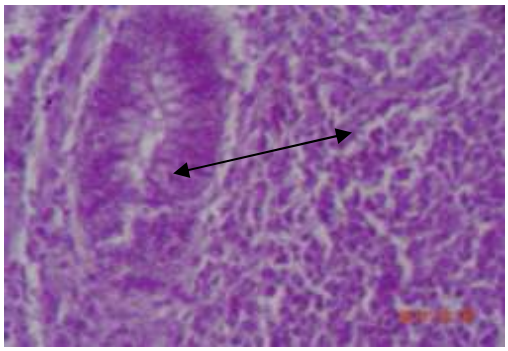

Fig. (9) Histological section in the cecal tonsil of 2nd group shows hyperplasia of goblet cell in the epithelial tissue with mononuclear cell infiltration in sub epithelial layer ⟷ (H & E stain 40X).

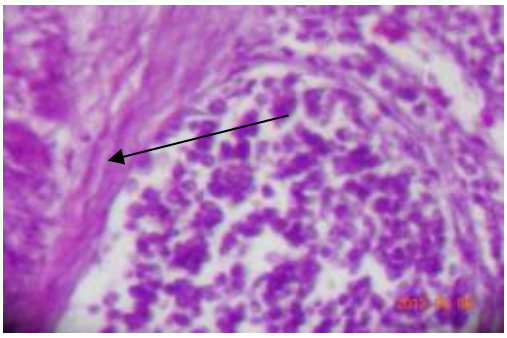

Fig. (10) Histological section in the Harderain gland of control group shows no clear lesion ⟶ (H & E stain 40X)

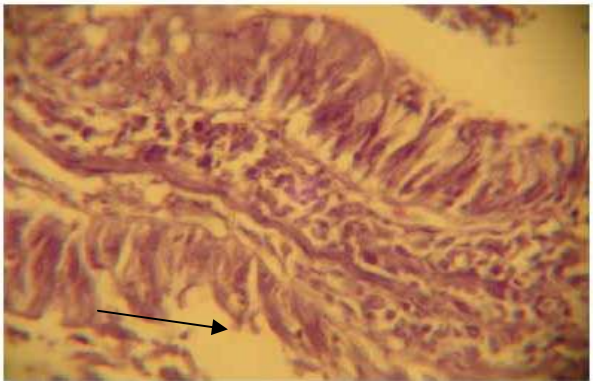

Fig. (11) Histological section in the cecal tonsil in control positive group shows inflammatory cells particulary heterophils in subepithelial layer sloughing of the epithelail cells ⟶ (H&E stain 40X)

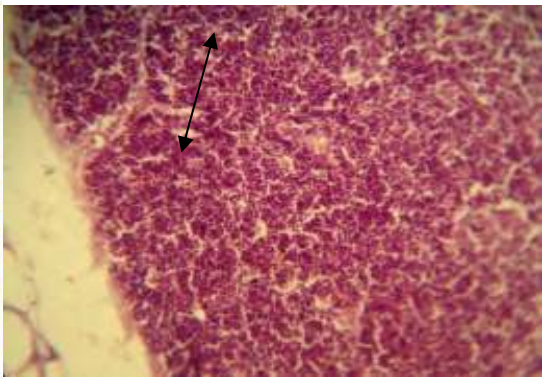

Fig. (12) Histological section in the thymus of 2ed group shows marked hyperplasia of lymphoid tissue ⟷ (H&E stain 10X).

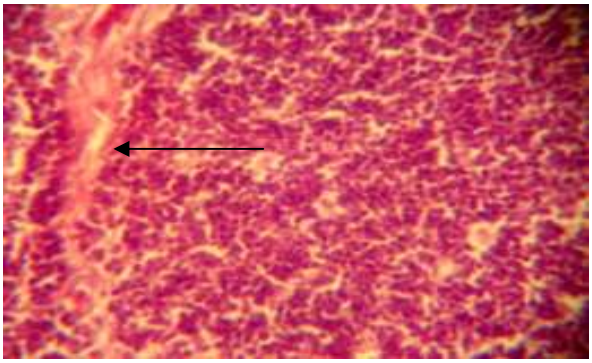

Fig. (13) Histological section in the thymus of 1st group shows moderate hyperplasia of lymphoid tissue with moderate thickness in the capsular region → (H & E stain 10X).

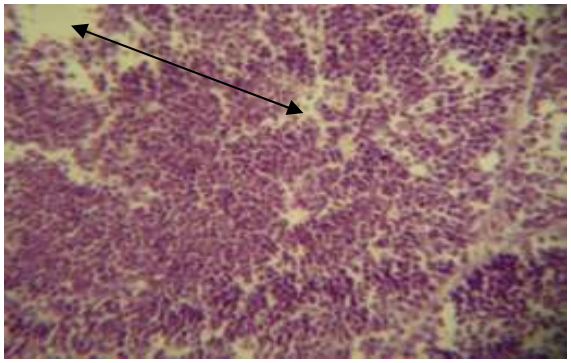

Fig. (14) Histological section in the thymus of control reveal marked depletion of lymphoid tissue ←→ (H&E stain 10X).

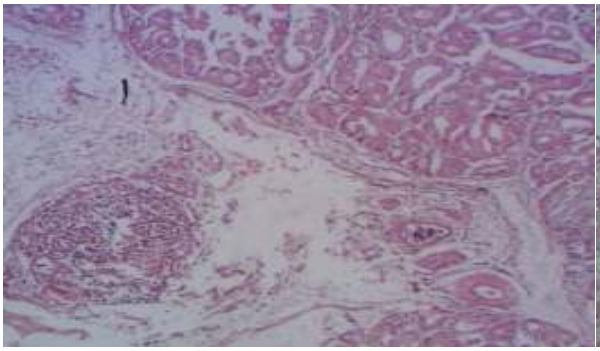

Fig. (15) Histological section in the Harderian gland of 1st group shows vacculation of epithelial lining cell with mononuclear cell infiltration and focal hyperplasia of epithelial lining cell of the gland (H & E stain 10X)

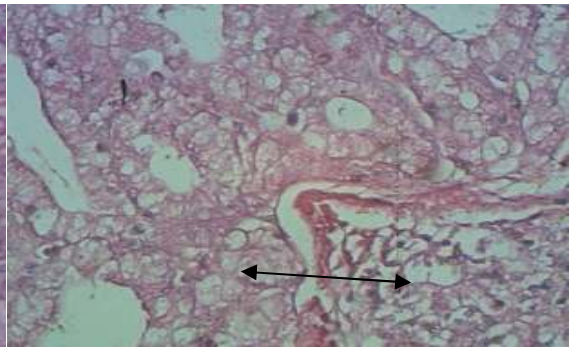

Fig. (16) Histolgical section in the Harderain gland of 2nd group shows focal hyperplasia of epithelial lining cells with mononuclear cells aggregation in subepithelial lining cells ←→ (H & E stain 40X).

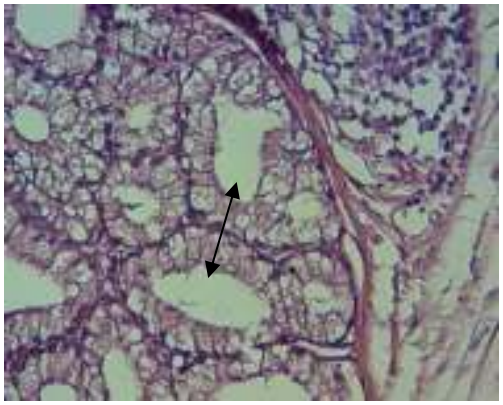

Fig. (17) Histological section in the Harderain gland not treated and challenged by *E.coli* showed sever vacculation in the acini ←→ (H & E stain 40X)

Discussion

The ability of the intestinal microbiota that contribute to the health of the host is well documented; however, the exact mechanisms by which the microbiota exerts its effect remain speculative. There is increasing evidence that through iteraction with innate immune system, probiotic bacteria are capable of modulating immune responses (15). Although the immunomodulatory activities of probiotic bacteria are not fully understood, it has been demonstrated that probiotic bacteria taken orally can increase cytokine levels in the serum in bacterial species-specific manner (16,17). and it is generally accepted that this is one of the ways in which probiotic bacteria alter the

adaptive immune response. In our study we observed that the 2nd group reveal extensive lymphoid hyperplasia as compare with other groups, it is indicative to the systemic immune response which is attributed to the ability of probiotic bacteria to enhance the ability of dendritic cells for antigen presentation (18) or changes in T-cell populations and functions leading to an increase in regulatory T-cells or polarization toward Th1 or Th2 responses (19). Also natural antibodies in GALT were enhanced following probiotic treatment (20). So chicken humoral immune response are induced in the secondary lymphoid organs, such as Harderain gland and cecal tonsils (21). In other hand, after challenge the 2nd group revealed a good protection as compared with the 1st group and this was attributed to use of probiotic, which is regarded as antibacterial and immunostimulant in controlling the intestinal phathogenic bacteria (22). Exposure to high environmental temperature for extended period will suppress the humoral immune response of chicken, cellular immunity is also suppressed by prolonged exposure to temperature in excess of $36C^0$, this effect is mediated through T-cell or regulatory amplifier cell response (23) so (22) who conducted in experimental treatments by addition of medical herbs (neem leaves), probiotic in broiler diet was efficient in controlling pathogenic bacteria in intestinal chicken especially *E.coli* improving broiler performance under heat stress condition and reduce the deleterious effects of heat stress, also there are researchers studies use probiotic as prophylaxis may show positive effect in broiler (24) and in layer (26). *L.acidophilus* was there for effective as therapeutic and prophylactic against for reducing mortality when chicks were administrated pathogenic *E.coli* (26). This bacteria produce lactic acid which alter the PH of chicken gut making it improper media for harmful bacteria such as pathogenic species of *E.coli* (27). Also produce specific antibacterial compounds such as hydrogen peroxide (28) and compete with other microbes for adhesive site (29). The protective percent in 3rd group which remained two chicks alive may be attributed to the effect of IgY which improved intestinal health and immune responses in broilers challenge (30). The histopathological changes in the thymus of the 1st and 2nd groups post infected, indicated an enhancement effect of probiotic on immune system of broiler was detected, the positive effect related to stimulate the lymphatic tissue (31), So this agree with (32) who showed an increased relative weight of thymus in probiotic treatment group at 28 and 42 days of age as compared to the control group. In 3rd group the marked depletion was due to infection of *E.coli* causing damage to the immune systems of the chicks (33). The Harderain gland is accepted to be a peripheral lymphoepithelail organ that together with the spleen, bursa of Fabricious and the cecal tonsils form a system of avian organs that determine both general and local immunity (34) also the age-related increase in plasma cell infiltration of Hg. (plasma-cellular development phase) has been observed in chicken (35) and increase plasma cell infiltration of chicken reached maximum concentration after 4wk of age (36). The vacculation of 3rd group may be due to abetter developed and branched blood supply that could be favorable factor for the spreading of the infection agent in the organ (37). Moreover; the probiotic treatment in the 2nd group exhibited higher increase in lymphocyte aggregation than 1st group.

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