Histopathological study of cecal tonsils in broilers exposed to heat stress after treatment with lactobacillus acidophilus

Balqees, H. A.; Ebtasam, J. A. and Nawals, J.
Department of Pathology and Poultry Disease, College of Veterinary Medicine, Baghdad University, Iraq.
E-mail: lbclg@yahoo.com
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Summary

This study was designed to investigate the effect of Lactobacillus spp on the histology of cecal tonsils in broiler chicks exposed to heat stress. Eighty broiler chicks were divided randomly into four groups equally and treated as follows: 1st group was administered orally with 1×10⁸/0.1 CFU/ml of (L.B.) at one day old, 2nd group was treated as 1st group but at 21 days old, and 3rd group (control positive), all three groups were exposed to heat stress (38-40°C) for period 10 days for three hours continuously, 4th group (control negative) was kept under natural condition. Organ samples from cecal tonsils were taken for histopathological test after 48 hrs., 120 hrs. and 240 hrs. of exposure to heat stress. The result revealed that most important lesions in 3rd group occur after 48 hrs. Cellular debri and marked cellularity (inflammatory cell) occur after 120hrs. was vacuolation and degeneration of epithelial layer cell. While the first 1st group after 48 hrs. showed moderate cellularity, after 120 hrs. showed marked lymphocyte proliferation and after 220 hrs. Increased goblet cell in the epithelial cell and 2nd group showed after 48 hrs. mild cellarity of lamina properia, and after 120 hrs. showed moderate lymphocytic hyperplasia. In conclusion exposure to heat stress caused destructive lesion in the cecal tonsil especially after 48 hrs. and 120 hrs. and gave lactobacillus acidophilus at 21 days more effective that at one day in reducing the effect of heat stress on histology of cecal tonsils.

Keywords: Cecal tonsils, Broilers, Heat stress, Lactobacillus acidophilus.

Introduction

Heat stress is a common wide problem in poultry production, high temperature reduce feed intake, body weight gain and increased feed conversion ratio (1). Heat stress decreased the immune response and ratio of bursa, thymus and spleen to body weight of the birds (2). Antibody response also reduged as well as phagocytic ability of macrophage in broiler under heat stress (3 and 4). Use of vitamin C and E with selenium (5 and 6), antibiotic and probiotic (7) as additives in feed was aimed to reduce the heat stress in the birds, the evidence is presented showing that treatment with probiotic protection occurs whether the disease is induced by an environmental, microbial or nutritional stresses (8). Probiotics are defined as live microorganisms like (Lactobacillus acidophilus) when administrated in adequate amounts confer a healthy effect on the host through improvements to the intestinal microbial balance (9), improve immunity; live weight gain, rates of feed conversion and decreased mortality of broiler (10 and 11). The intestinal microbiota is in constant contact with cells of gut-associated lymphoid tissue (GALT), which includes professional antigen-presenting cells, B-cells, T-cells and intestinal epithelial cells. In the case of the chicken, cecal tonsils are the major lymphoid tissues within the GALT, they are located at the proximal region of the cecum (12) chicken cecal tonsils contain germinal centers and IgA, positive B-cells (13) The interaction of cells from the GALT with commensal bacteria is thought to be critical for establishment and maintenance of intestinal homeostasis.In this study, the aim was to determine the probiotic (lactobacillus acidophilus) effect and heat stress on the histology of cecal tonsils of broiler in different periods.

Materials and Methods

The experiment was carried out in the poultry house of Pathology and Avian diseases department/ College of Veterinary Medicine/ University of Baghdad. Poultry house was cleaned and disinfected before chicks admittance, chicks were provided with free
access to water and feed. *Lactobacillus acidophilus* isolate was obtained from College of Agriculture/ University of Baghdad; Dr. Bushra Saady with concentration 1×10⁸/0.1 CFU and gavages (1ml) into crop.

Eighty one day old Rose broiler chicks from a commercial broiler breed (Rose, Jordan origin) were divided randomly into four groups 20 chicks to each one as following: 1st group was given *Lactobacillus acidophilus* in one day old only, 2nd group was given *Lactobacillus acidophilus* in 21 days old only, 3rd group was not given *Lactobacillus acidophilus* but this group and the groups 1st and 2nd were carried out under heat stress condition (38-40 °C) for 3 hrs. in a day for long a period of 10 days, starting in 21 day old, 4th group was given normal saline (-ve control). Specimens were taken from internal organs including cecal tonsils after (48 hrs., 120 hrs. and 240 hrs.) after exposure to heat stress. These tissues were fixed into 10% buffer formaldehyde solution immediately after removal. The processing was routinely done and then slides were stained with H and E stain (14 and 15) and histopathological changes were observed under light microscope.

**Results and Discussion**

The histopathological changes in the cecal tonsils occur after 48, 120 and 240 hrs. of exposure to heat stress for all groups of this study, In 1st group after 48 hrs. the section showed moderate inflammatory (mononuclear) cells infiltration in the subepithelial layer. (Fig. 1). After 120 hrs., the section showed marked lymphocyte proliferation in the submucosa (Fig. 2) and after 240 hrs. it showed moderate cellularity with increase goblet cells in the epithelial layer (Fig. 3). In 2nd group after 48 hrs. showed mild cellularity of lamina propera (Fig. 4). After 120 hrs. showed moderate lymphocyte hyperplasia in the lamina propria (Fig. 5), while after 240 hrs. no clear lesion was seen. (Fig. 6). In 3rd group (control positive) after 48 hrs. was cellular debris in their lumen and marked cellularity of the lamina propera with vacullation of lining epithelial cells (Fig. 7) after 120 hrs. showed inflammatory cells infiltration in the subepithelial layer with vacuolar degeneration of epithelial layer cells (Fig. 8).
and degeneration of epithelia cells and these effect result from that the heat stress reduced blood flow to the periphery resulting in local deprivation of oxygen and energy (16). While after 240hrs. it showed increased mononuclear cells infiltration in the subepithelial layer and hyperplasia of epithelial cell and marked cellularity of lamina propria (Fig. 9).

Figure 5: Intestine of 2nd group after 120hrs. heat exposure showed moderate lymphocytic hyperplasia in the sub-epithelial layer ( ) and moderate cellularity of lamina propria ( ) (H and E stain 100X).

Figure 6: Intestine of 2nd group after 240hrs. heat exposure no clear lesion seen. (H and E stain 400X).

Figure 7: Intestine of 3rd group after 48hrs. heat exposure showed ( ) cellular debress in their lumen and marked cellularity of the laminapropria ( ) with vacuolation of lining cells ( ) (H and E stain 400X).

While after 240 hrs. it showed increased mononuclear cells infiltration in the subepithelial layer and hyperplasia of epithelial cell and marked cellularity of lamina propria (Fig. 9). The 4th group (control negative) revealed no clear lesion (Fig. 10). Present result Indicated that the effect of heat stress was seen in 3rd group (control positive) after 48hr and 120 hrs. of exposure to heat stress by presence of cell debris, vacuolation

Figure 8: Histological section in the intestine of 3rd group after 120hrs. heat exposure shows inflammatory cells infiltration in the subepithelial layer ( ) with vacuolar degeneration of epithelial layer cell. (H and E stain 400X).

Figure 9: Intestine of 3rd group (control positive) after 240hrs. heat exposure showed ( ) marked mononuclear cell infiltration in the sub epithelial layer and around mucosa gland which showed of epithelial lining cell and marked cellularity of hyperplasia Lamina propria ( ) (H and E stain, 400X).

Figure 10: Intestine of 4th group (control negative) revealed no clear lesion. (H and E stain, 400X).
The 4th group (control negative) revealed no clear lesion (Fig. 10). Present result indicated that the effect of heat stress was seen in 3rd group (control positive) after 48 hr and 120 hrs of exposure to heat stress by presence of cell debris, vacuolation and degeneration of epithelia cells and these effect result from that the heat stress reduced blood flow to the periphery resulting in local depravation of oxygen and energy (16), this deprivation generates reactive oxygen species with in gut cell leading to impaired cell structure and membrane integrity. Also these result linking to reduced level of thyroid hormones during heat stress are believer to have enterotrophic effect (17) the above lesion was not presented in 1st and 2nd groups and this reflected protective effect of given probiotic lactobacillus acidophilus at one day old and 21 days old improvement or protected the epithelial lining from effect of heat stress and this may be attributed to the increased circulating level of thyroid hormone (18), activation of cell mitosis (19) and increase vascularity of villi in response to the intestinal microbes (20). Stress induced damage to the intestinal mucosa releases pro-inflammatory cytokines leading towards proliferation (21), and infiltration of more inflammatory cells.

In the present study there was marked cellularity and infiltration of inflammatory cell in all period in 3rd group while in 1st group there was mild cellularity and reduction in an inflammatory response triggered by heat stress and this result may be attributed to probiotics-induced reduction in the level of pro-inflammatory cytokines (21) and this more pronounced after 48 hrs. also the result revealed increase goblet cells in the epithelial layer of cecal tonsils of 1st group compared with 3rd group the goblet cells acts as a barrier between luminal pathogens and under lying vascular supply (22). The heat stress-induced perturbation in the integrity of intestinal epithelium reduces its protective function by loss of mucus producing (goblet cell). The observed increase in goblet cell due to supplementation of probiotic (lactobacillus) can be attributed to their mucin gene regulation acceleration of differentiation and immunostimulatory effect of probiotics (23), the results observed lymphocytic hyperplasia in the subepithelial layer of 2nd group after 120 hrs. compared with 3rd group (control positive) result from the gut colonization by lactobacillus which lead to develop gut associated lymphoid tissue by activation of lymphocyte (24), and probiotic stimulate protective immune response sufficiently to enhance resistance to microbial pathogen (25), while in 3rd group exposure to heat stress showed reduced lymphocyte with vaculation and degeneration of lymphocyte specially after 48 hrs. and 120 hrs. These were related to that heat stress stimulates the release corticosteron and initiated lipid peroxidation in cell membrane of T and B lymphocyte (26). These result agree with (27) who found that dietary supplementation of lactobacillus may be helpful in alleviation some of the detrimental effects of heat stress on microsturture of the broiler gut. From these results we may conclude that heat stress cause sever histopathological changes after 48 hrs. and 120 hrs. in the cecal tonsil, and giving lactobacillus at 21 days old reduced these effect and activation of the lymphocytic tissue in the cecal tonsils, and in less degree giving lactobacillus at one day old reduced effect of heat stress by increasing goblet cell.

References


دراسة نسجية مرضية للوز الاعورين في دجاج اللحم بعد تعرضه للعصيات اللبنية والإجهاد الحراري
بلقيس حسن علي و ابتسام جواد علي و نوال صالح جعفر
فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة بغداد، العراق.
E-mail: lbc_lg@yahoo.com

الخلاصة
اُجريت الدراسة لمعرفة مدى تأثير العصيات اللبنية والإجهاد الحراري على نسيج لوز الاعورين في افراخ دجاج اللحم، حيث استعملت ثمانية فرخة قسمت عشوائيا إلى أربعة مجموعات كالآتي: المجموعة الأولى أعطيت العصيات اللبنية بجرعة (8×10⁸ CFU/ml) فمويا، المجموعة الثانية أعطيت كالمجموعة الأولى ولكن بعد 21 يوم، المجموعة الثالثة اعتبرت موجبة، افراخ المجاميع الثلاثة عرضت الى الإجهاد الحراري (83-04 م°) لفترة ثلاث ساعات متواصلة لكل يوم ولأمسية عشرة أيام، بينما اعتبرت المجموعة الرابعة موجبة سالبة وضعت في ظروف طبيعية واخذت عينات من لوز الاعورين لإجراء الفحص النسجى بعد 48 و 120 و 240 ساعة من تعرضها. أظهرت النتائج ان من اهم الافات النسجية في المجموعة الثالثة كانت وجود حطام خلايا وزيادة مميزة في اعداد الخلايا (الخلايا التهابية) بعد 48 ساعة و 214 ساعة و 104 ساعات من التعرض. بينما أظهرت المجموعة الثانية بعد 03 ساعة وجود زيادة قليلة في اعداد الخلايا (خلايا التهابية) بعد 214 ساعة. ونتيجة هذه الدراسة أن التعرض للاجهاد الحراري يسبب تأثير تخريبي لنسج لوز الاعورين، بينما أظهرت المجموعة الأولى أدنى تأثيرات على نسيج لوز الاعورين. الكلمات المفتاحية: لوز الاعورين، دجاج اللحم، الإجهاد الحراري، العصيات اللبنية.