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A MORPHOLOGICAL STUDY ON THE POTENTIAL ROLE OF SODIUM BUTYRATE IN THE PROTECTION OF THE SPLEEN IN HEAT-STRESSED BROILERS

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ABSTRACT

Heat stress (HS) is one of the major economic problems facing the world's chicken industry. Besides decreased growth performance, HS reduces immune function in chickens. The principal part of the immunological function is the lymphatic organ. The spleen is a secondary lymphoid organ in birds. Sodium butyrate (SB) is the sodium salt of butyric acid and is suggested to be used to relieve heat stress. The current study aimed to evaluate the effect of sodium butyrate on the spleen in heat-stressed broilers. A total of 150 healthy broiler chicks (1 day old) were randomly divided into 4 groups: 1) control group fed on basal diet (BD) and normal temperature; 2) heat stress (HS) group (BD) and exposed to HS (34◦C for 8 h/day for 7 days starting from 29 days old); 3) low sodium butyrate group (BD + 0.5 g/kg SB every day and exposed to HS and 4) high sodium butyrate group (BD + 2 g/kg SB every day and exposed to HS. The spleen was harvested at the end of the experiments (36 days old). Our results revealed that HS caused severe damages, such as noticeable atrophy and loss of organization in the white pulp and hemorrhage in both white and red pulps. A low dose of SB supplementation showed full protection of the spleen tissue. However, the high dose might cause an adverse effect. In conclusion, SB especially low dose ameliorated the effect of heat stress. Therefore, we suggest using SB to face the heat stress in broilers, however with low doses.

Keywords; spleen, Heat stress, Sodium butyrate, Immunity, Broiler.

BACKGROUND

The global rise in temperatures is one of the greatest environmental stressors that chicken producers face around the world. Because chickens lack the facility to scatter their body heat due to their feather covering and inadequate sweat glands, their thermoneutral zone (16–25°C) is particularly small. Moreover, high productivity, excessive stocking densities, and rapid growth rate make them the most sensitive livestock species to heat stress (HS) (Hirakawa *et al.*, 2020; Hu *et al.*,

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2022). Heat stress induces morphologic changes to the lymphoid organs of broiler chickens at the macroscopic and microscopic levels. These changes are reported in primary and secondary avian lymphoid organs (Bartlett *et al.,* 2003; Niu *et al.,* 2009; Lola *et al.,* 2016). The primary lymphoid organs are the thymus and bursa of Fabricius, while the secondary lymphoid organs are the spleen, mucosa-associated lymphatic tissue, Harderian gland, and bone marrow (Hamoda & Farag, 2018). The lymphatic organs are considered the cornerstone of immunological function in birds (Lan *et al.,* 2020). Heat-stressed chickens exhibit morphological changes and lymphoid depletion in the bursa of Fabricius, thymus, and spleen (Bartlett *et al.,* 2003; Niu *et al.,* 2009). The avian spleen is a secondary lymphoid organ essential in immune reactivity against viruses and bacteria (Akaichi *et al.,* 2022; Selim *et al.,* 2021). In birds, the spleen does not function as a storage site for blood cells but rather serves as a site for antibody production, purification of circulating blood, and facilitation of lymphocyte-antigen interactions. Additionally, it plays a role in the ingestion of immune complexes and damaged blood cells by macrophages **(**Aguanta *et al.,* 2018).

The heat stress causes multiple immune abnormalities in broiler chickens by impairing the developmental process and functional maturation of T and B cells in both primary and secondary lymphoid tissues (Hirakawa *et al.,* 2020). Nutritional modulation of immunological status via feed additives may have good benefits and provide a simple path to enhancing poultry health and productivity. However, dietary antibiotics increase the risk of developing resistance to antibiotics used to treat bacterial infections in animals, humans, and fish **(**Hu *et al.,* 2022; Zhang *et al.,* 2011). Butyrate or its sodium salts, as feed additive, are suggested for the optimum development of intestinal epithelium and gut-associated lymphoid tissues (Friedman & Bar Shira., 2005). Dietary sodium butyrate supplementation improved the development of the gastrointestinal tract by increasing the relative weight and length, as well as enhancing the immune response to the ND vaccine **(**Lan *et al.,* 2020**)**. Nutritional regulation of sodium butyrate may bring beneficial effects and provide a simple path for immune organ development. The former studies on the use of sodium butyrate, as feed additive, were focused on their growth performance, gut morphology, antimicrobial, immunomodulatory, and antioxidative capacities (Liu *et al.,* 2014; Song *et al.,* 2017; Zhang *et al.,* 2011). However, limited work has reported the effects of SB on the modulation of undesirable morphological changes in the spleen of the stressed chicken. We assume that dietary SB may play an immune-enhancing role by protecting the affected lymphoid organs. Therefore, histological analyses of the changes in the lymphoid organ under HS conditions should be evaluated to develop a new production plan that accommodates heat-stressed broiler chickens to lessen the impact of infectious diseases and improve productivity. So, this work aimed to study the potential role of sodium butyrate in modulating the immunosuppressive effects of the spleen in heat-stressed broilers.

MATERIALS AND METHODS

The experiment was conducted in the Poultry Research Farm of the Faculty of Agriculture, Assiut University; (approved ethically, 06/2023/0110, by the Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt). This work was funded by the Egyptian Ministry for Scientific Research, the Science, Technology & Innovation Funding Authority (Grant No. 39382). The experimental diets were formulated from commercially available ingredients in three stages (starter, grower and finisher). The study is reported following ARRIVE guidelines.

The experimental design

A total number of 150 one-day-old broiler chicks (*Gallus gallus domesticus*) (Cobb 500, obtained from a local commercial hatchery) were divided randomly into four groups. Birds were housed in experimental chambers under the recommended optimal conditions of temperature, humidity and ventilation. All precautions for dealing with animals were taken. The birds had free access to fresh tap water and were fed ad-libitum on a commercial ration, including (23% CP + 2950 kcal ME/kg) for the starter and 21% CP + 3100 kcal ME/kg) for the grower, which covered the starting and growing requirements, respectively.

- Group (1): The normal control group lived at normal temperature (24˚C) and received a basal broiler diet.
- Group (2): The heat stress group, received a broiler diet and was exposed to 34˚C from 8 am to 4 pm (8 h/day), and exposed gradually to 24˚C from 4 pm to 8 am every day, starting from day 29 to day 35 of age.
- Group (3): The low sodium butyrate group, received a broiler diet + 0.5 g/kg SB. They were exposed to the same heat scheme of group 2.
- Group (4): The high sodium butyrate group, received a broiler diet + 2 g/kg SB. They were exposed to the same heat scheme of group 2.

Experiment duration: 5 weeks

Sample collection:

The birds were sacrificed by slaughtering at the end of the study on the 36th day. Tissue samples from the different parts of the spleen were collected and prepared for the light microscopic examination.

Paraffin-embedded samples:

The tissue samples from the spleen were collected from the slaughtered birds and fixed in a 10% buffered formalin solution for 48 hours. Tissues were dehydrated by dipping through a series of ethyl alcohols of increasing concentrations (from 70% to absolute), cleared with xylene, and embedded in paraffin blocks. The samples were then paraffin-embedded and 5 microns thick sections were cut and stained with hematoxylin-eosin (Harris, 1900), for general examination. The slides were examined using a light microscope [Olympus CX41 light microscope equipped with a digital Olympus camera (C-5060, Japan)] and digitized images.

Semi-thin sections:

The tissue samples were collected from the slaughtered birds and fixed in a mixture of 2.5 % glutaraldehyde and 2.5 % paraformaldehyde (pH 7.2). After careful cleaning in phosphate buffered saline (pH 7.4), the specimens were post-fixed in 1% osmium tetroxide for two hours at room temperature, and then rinsed in 0.1 M PBS overnight. Samples were directly dehydrated through increasing levels of alcohol:

70% for at least an hour, 90% for 30 minutes, and twice 100% for 15 minutes each. Following having penetrated with a 1:1 mixture of regular Spurr's resin and 100% acetone (overnight), 2:1 (during the second day), and two changes of pure resin (12 hours each), samples were implanted in pure Spurr's resin. Slices cut using an ultramicrotome Ultracut E (Reichert-Leica, Germany) into (1 μm) semi-thin sections were stained with toluidine blue and examined using a light microscope.

RESULTS

Micro morphological Changes in the spleen;

Microscopically, the normal broiler spleen consists of white pulp (ellipsoid surrounded by PELS) and red pulp consists of (blood sinusoid and splenic cord) and is enclosed by a thick fibrous capsule, from which few trabeculae are extended from it inward. The white pulp is made of a network of reticular cells and reticular fibers. Small, medium, and large-sized lymphocytes and plasma cells were evenly dispersed throughout the white pulp. The red pulp was less defined and scattered haphazardly among the white pulp of the spleen. Each red pulp consists of venous sinuses and an anastomosing cord of blood, lymphocytes, macrophages, and reticular cells. Red blood cells make up the majority of the red pulp, (Fig. 1A & 2). The lymphatic cells of the spleen include B lymphocytes and T lymphocytes; the B lymphocytes multiply and undergo differentiation in the lymphatic nodules. In addition, they formed lymphoid tissue that encircles the branching penicillary capillaries called the peri-ellipsoid lymphocyte sheaths (PELS). Mast cells were rounded with rounded nuclei and contained numerous rounded granules of different sizes in their cytoplasm. Monocytes are large agranulocytes with distinctly indented or C-shaped nuclei. Macrophages with kidney-shaped nuclei contained phagocytic materials (inclusions) of different shapes and sizes (Fig. 3).

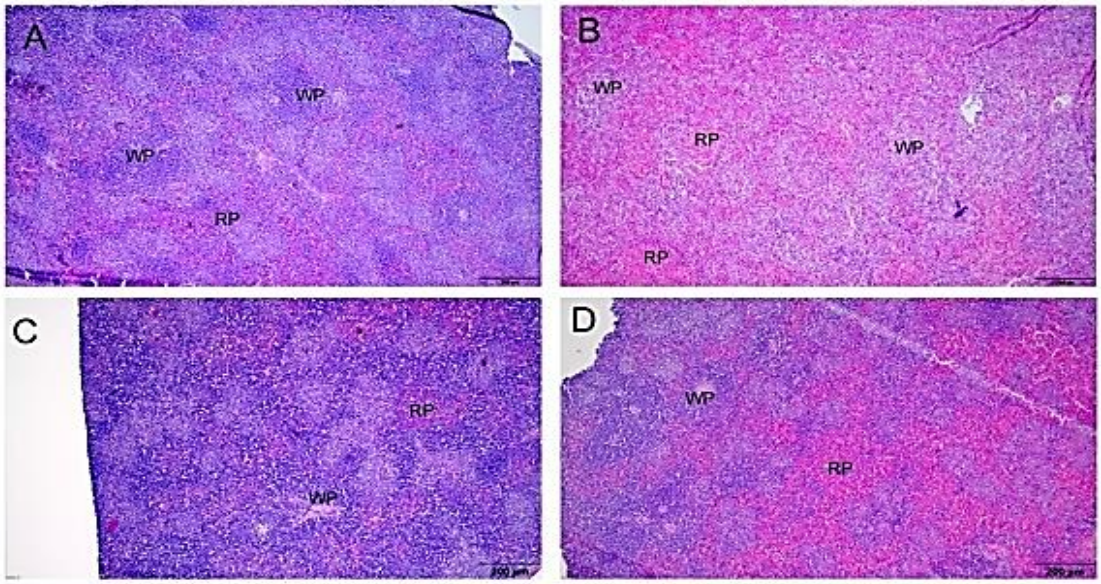

Figure (1): The spleen from the control, heat-stressed chickens and the supplemented groups with sodium butyrate (SB); A) control group showing the normal structure of spleen, white pulp (WP) and red pulp (RP). B) Spleen of heat stress (HS) group showing severe depletion in WP and a pronounced hemorrhage. C) Spleen of HS supplemented with low SB, showing an almost or nearly normal microscopical structure of WP & RP. D) Spleen of HS group supplemented with high SB showing slight depletion of WP and moderate congestion in RP. (Hx&E stain).

Heat stressed group:

The spleen of the heat-stressed broiler chickens showed degenerative changes, when compared to the control group, such as degenerative alterations, shrinkage, distortion in the architecture of white pulp (depletion in PALS and PELS), and apoptosis in lymphocytes (Fig. 1B & 4). Also, enlarged red pulp is present due to hemorrhage, congestion, and abnormal aggregation of large vesicles. It presents cellular degeneration and vacuolation in their cytoplasm. Moreover, abnormal mast cells, plasma cells and macrophages were observed in the white and rep pulps (Fig. 5).

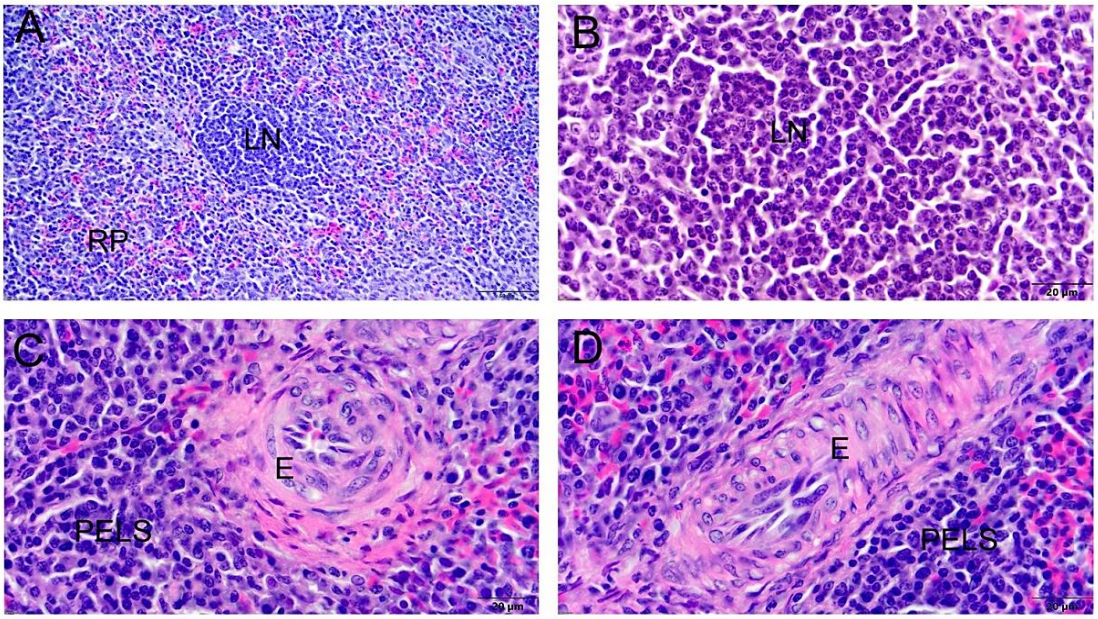

Figure (2): Photomicrographs of the spleen collected from the control group showing the normal structure of the spleen; normal white pulp (Ellipsoid and PELS), lymphatic nodules and normal red pulp (RP), Ellipsoid (E), periellipsoid lymphocyte sheaths (PELS), lymphatic nodule (LN). (Hx&E stain).

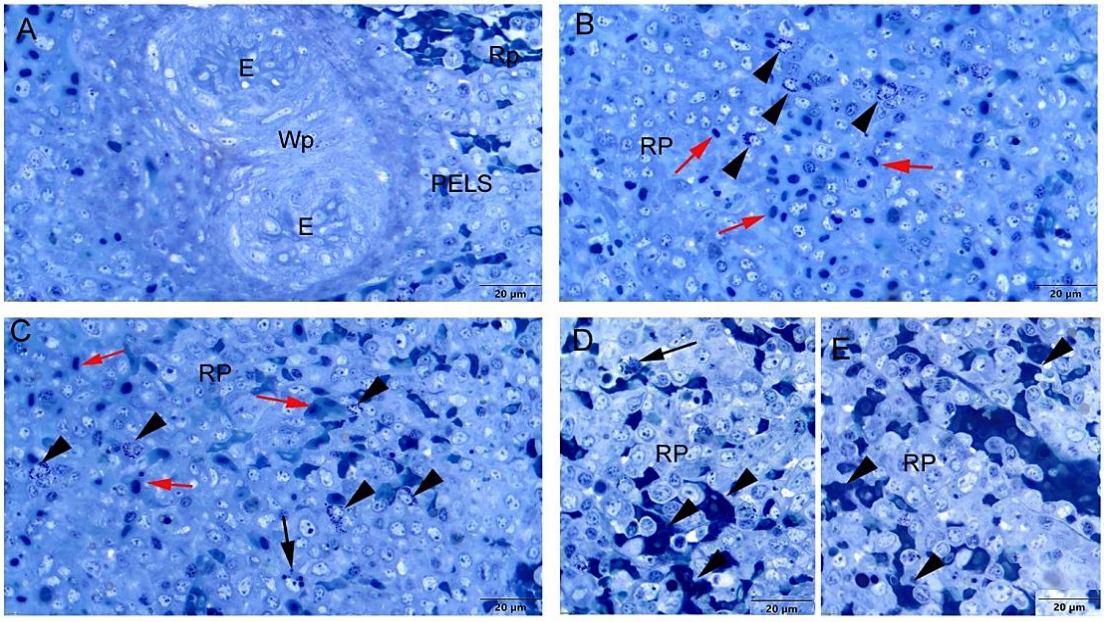

Figure (3): Semi-thin section of the spleen collected from the control group; A) white pulp (Wp), Ellipsoid (E) and (PELS). B) red pulp, mast cell (arrowhead), red blood cell (red arrow). C) mast cell (arrowhead), macrophage (black arrow), Red blood cell (red arrow). D) macrophage (arrow), red blood cell (black arrowhead). E) Red blood cell (black arrowhead). (toluidine blue stain**).**

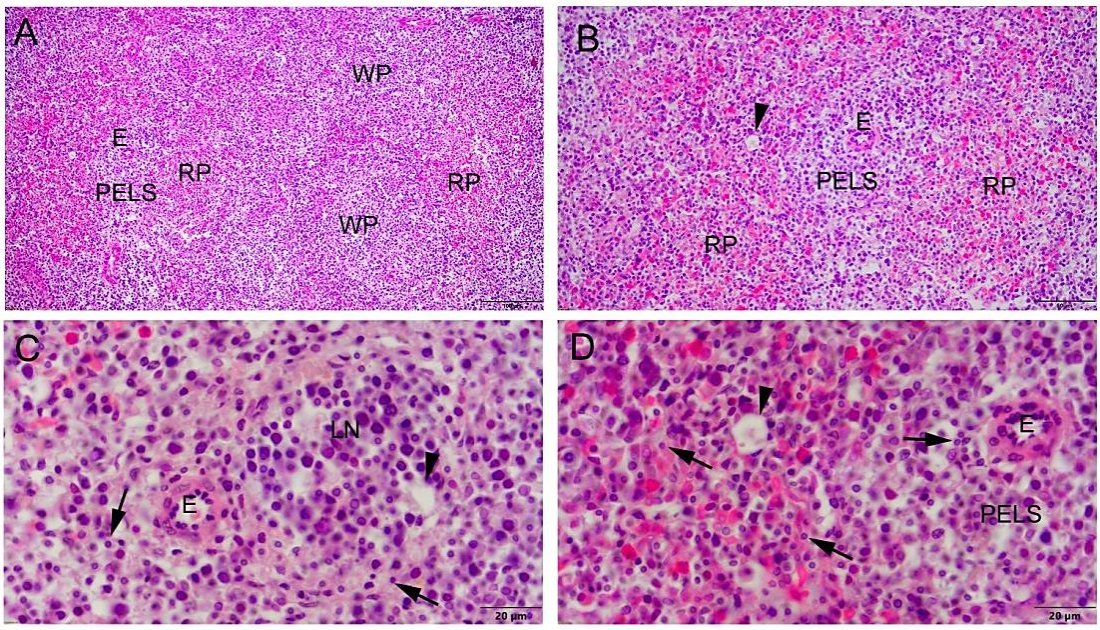

Figure (4): Photomicrographs of the spleen collected from the heat-stressed group; most specimens showed; hemorrhage and hyperplasia in the red pulp. Hemorrhage, degenerative changes, atrophy and loss of organization in the white pulp (depletion in PELS), and apoptosis in lymphocyte (arrow), vacuolation (arrowhead), white pulp (WP), red pulp (RP), Ellipsoid (E), periellipsoid lymphocyte sheaths (PELS), lymphatic nodule (LN). (Hx&E stain).

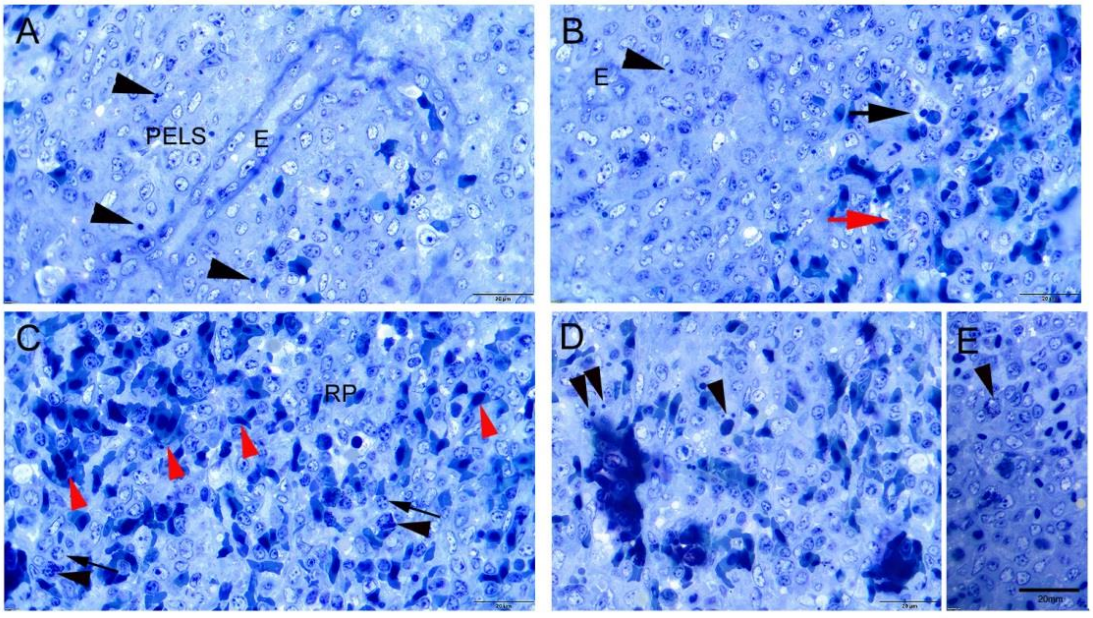

Figure (5): Semi-thin section of the spleen collected from the heat-stressed group: A & B) presented depletion in white pulp cells (ellipsoid and PELS), apoptotic cells (arrowheads), macrophage (black arrow), abnormal aggregation of large vesicles (red arrow). C) red pulp presented hemorrhage, red blood cells (red arrowhead), abnormal plasma cell (arrow), and mast cell (black arrowhead). D) red pulp with hemorrhage, apoptotic cells (arrowhead). E) mast cell in the red pulp (arrowhead). (toluidine blue stain**)**.

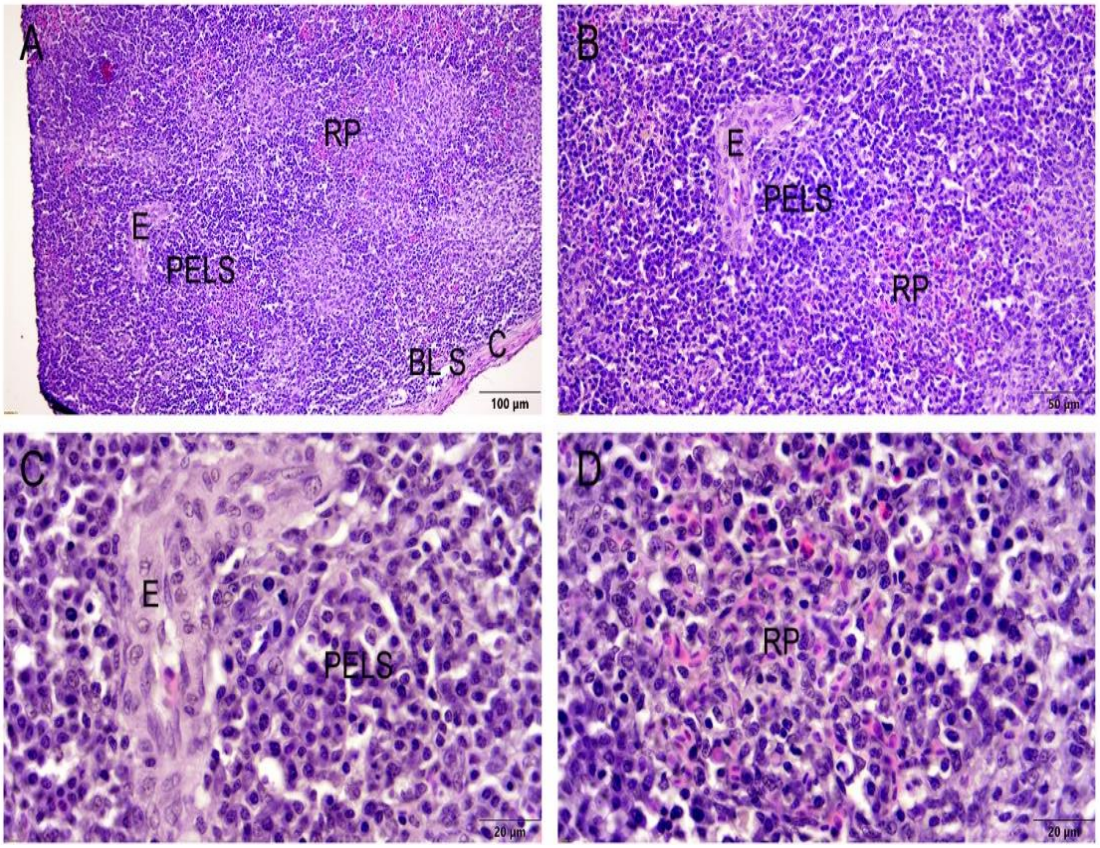

Figure (6): Photomicrographs of the spleen collected from the heat-stressed group supplemented with low sodium butyrate; different structures of the spleen appeared almost normally which include normal white pulp with no hemorrhage, no apoptosis and no depletion, red pulp (RP), Ellipsoid (E), periellipsoid lymphocyte sheaths (PELS), lymphatic nodule (LN), Blood vessels (BLs), capsule (C). (Hx&E stain).

In the heat-stressed group supplemented with Low Sodium Butyrate:

The spleen appeared almost normally and showed normal architecture in white pulp (Artery and PALS), (ellipsoid and PELS), lymphatic nodule and red pulp, compared to the HS group. No degenerative changes, no depletion, no hemorrhage, and no apoptosis (Fig. 1c & 6). Moreover, a large number of immune cells, like normal plasma cells, neutrophils, stem cells, macrophages, monocytes, mast cells, and eosinophils were noticed. In addition, the cellular structure of the spleen presented frequent mitotic division (Fig. 7,8).

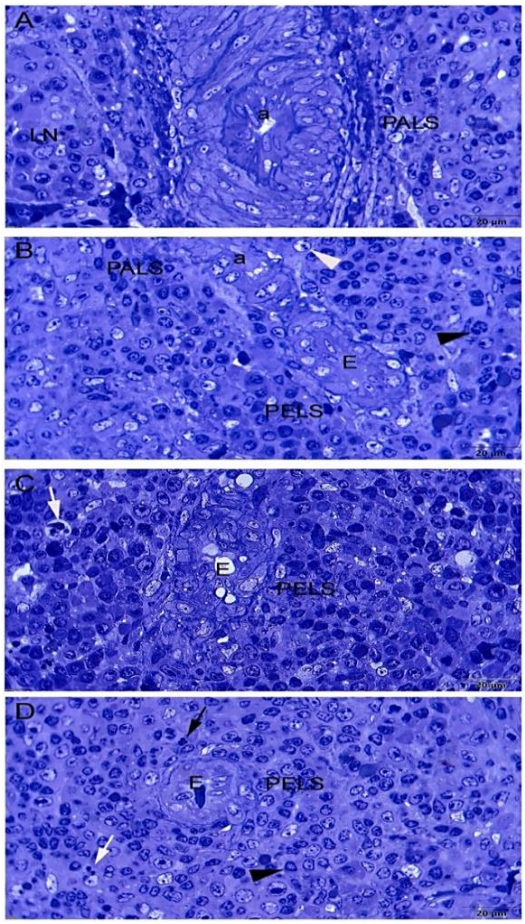

Figure (7): Semi-thin sections of the spleen collected from the heat-stressed group supplemented with low sodium butyrate showed; A-B) appeared normal Artery (a), and PALS, Ellipsoid and PELS, lymphatic nodule (LN), neutrophile (black arrowhead), stem cell (white arrowhead). C-D) Ellipsoid (E) and PELS, macrophage (white arrow). D) plasma cell (arrowhead), apoptotic body (black arrow). (toluidine blue stain**)**.

In the heat-stressed group supplemented with high Sodium Butyrate:-

The spleen presented less depletion in the white pulp (Artery and PALS), (Ellipsoid and PELS), lymphatic nodule, vacuolation in the wall of the blood vessels and moderate red pulp congestion (Fig. 1D & 9). Some stem cells and immune cells exist in the splenic cord, such as macrophages. In addition, both normal and abnormal plasma cells and mast cells were also observed (Fig. 10).

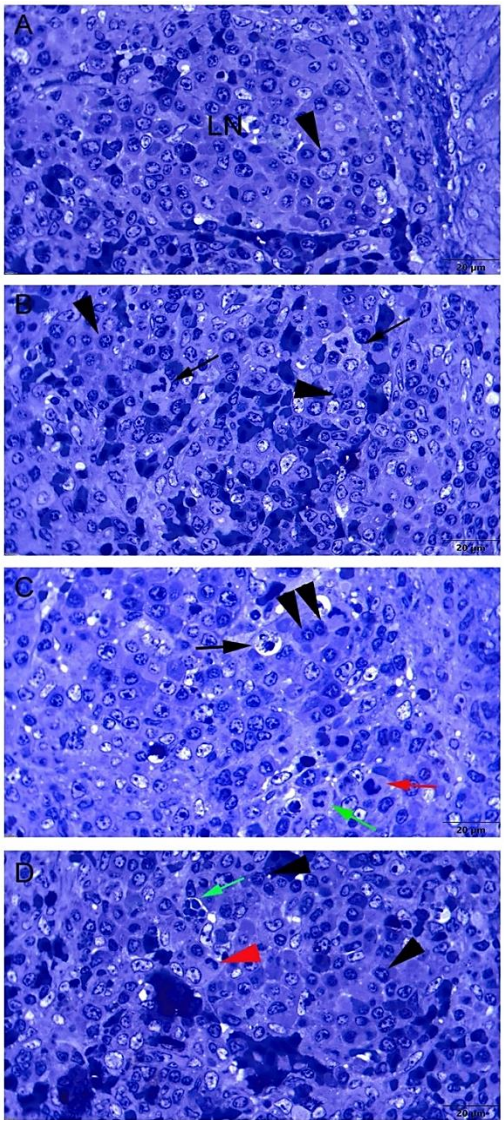

Figure (8): Semi-thin section of the spleen collected from heat-stressed group supplemented with low SB showing; A) normal white pulp (lymphatic nodule) with no degenerative changes, plasma cell (arrowhead). B) normal red pulp, plasma cell (arrowhead), neutrophil (arrow). C) plasma cell (arrowhead), monocyte (red arrow), mitotic division (green arrow), macrophage (black arrow). D) plasma cell (arrowhead), phagocytosed RBCs (green arrow), eosinophil (red arrowhead). (toluidine blue stain**)**.

DISCUSSION

The spleen is a secondary lymphoid organ that plays a vital role in preserving immune response. Certain substances improve early bird

immunity. For instance, broiler chicks given doses of organic acids like fumaric and citric acid showed mild to moderate expansion of the lymphoid follicles in the spleen, indicating an improved immune response (Attia *et al.*, 2018).

In the present investigation, we examine the effects of heat stress on the spleen in broilers, as well as the potential mitigation impact of two different concentrations of sodium butyrate on the level of the components of the cells by using histology and ultrastructure tech niques. In the current study, the microscopic examination of the spleens from the control group showed normal white pulp and red pulp. Various problems, such as stresses or pathogenic reasons, can impact the avian spleen. We found significant microscopic alterations in the heat stress group after exposure to higher temperatures, such as congestion, hyperplasia in the red pulp and hemorrhage, degenerative changes, depletion, atrophy and loss of organization in the white pulp, accompanied by a decreased immune cell number, compared to the control group. Our results agreed with Hirakawa *et al.* (2020), who reported that the weight of the spleen in broiler chickens under heat stress conditions was severely reduced, and histologically demonstrated improper destruction of the encapsulated germinal center (GC). A considerable rise in lymphoid depletion was also reported in splenic tissues taken from heat-stressed birds at 21 days of age (Aguanta *et al.*, 2018). The spleen can be impacted by some infections, such as the new type gosling viral enteritis (NGVE) in goslings.

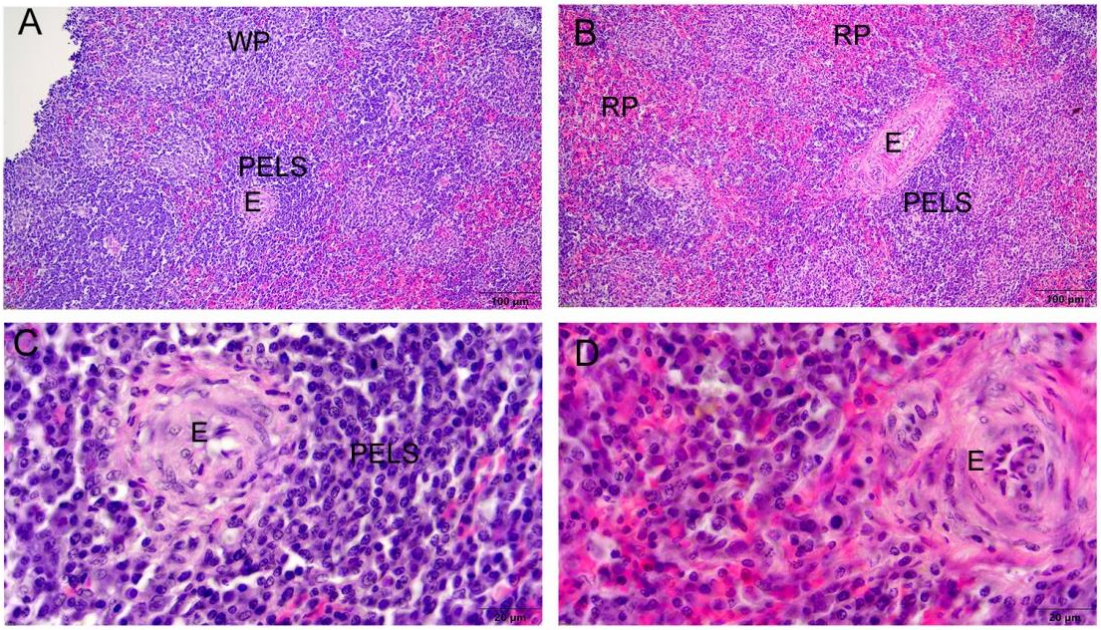

Figure (9): Photomicrographs of the spleen collected from the heat-stressed group supplemented with high SB; showed less depletion in the white pulp, less apoptosis in the cell and moderate congestion in the red pulp. (Hx&E stain).

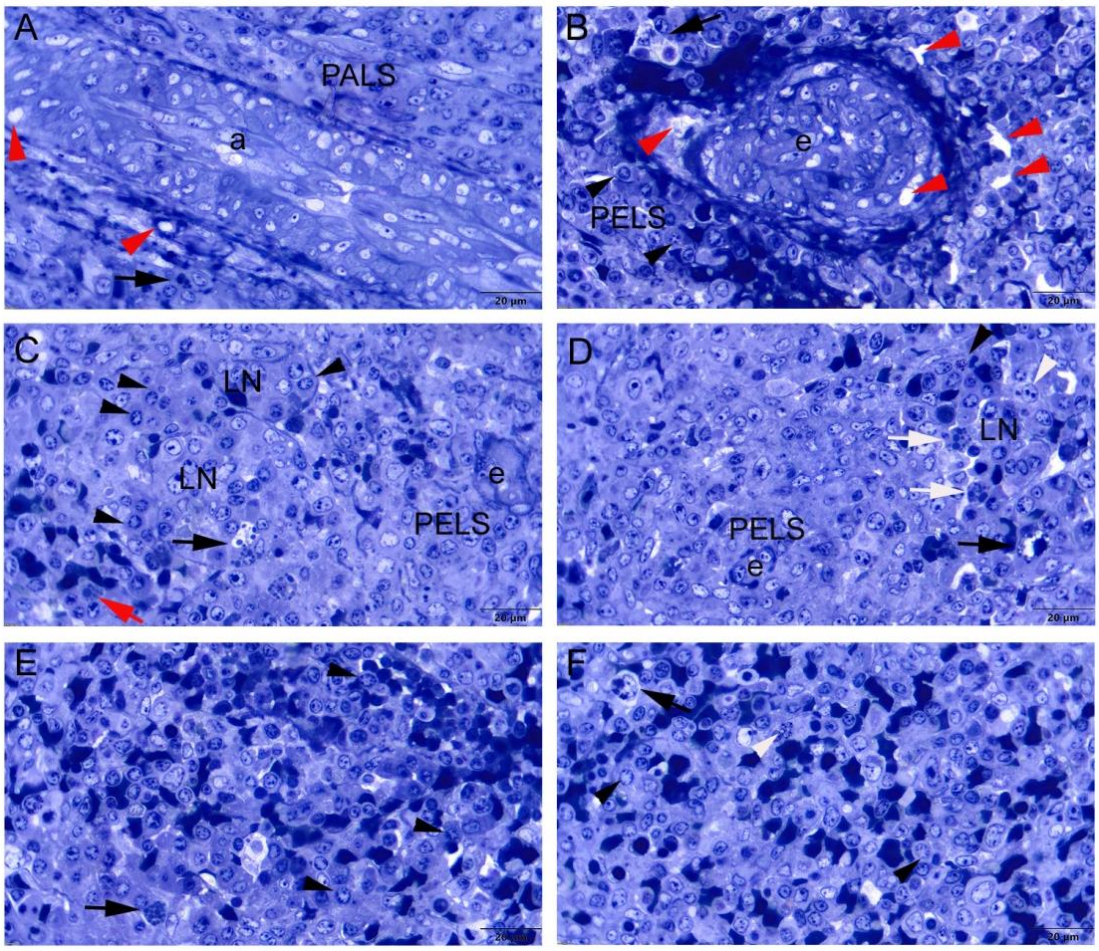

Figure (10): Semi-thin section of the spleen collected from heat-stressed group supplemented with high SB showing; A) white pulp, vacuolation in the wall of the artery (red arrowhead), PALS, apoptotic bodies (black arrow)**.** B) white pulp, vacuolation in the wall of the ellipsoid (red arrowhead), PELS, macrophage (arrow), plasma cell (black arrowhead). C) slight depletion in white pulp (ellipsoid and PELS), lymphatic nodules (LN), plasma cell (arrowhead), macrophage (black arrow), and mast cells (red arrow). D) Slight depletion in white pulp (ellipsoid and PELS), lymphatic nodules (LN), macrophage (black arrow), mast cell (black arrowhead), apoptotic cells (white arrow), and stem cell (white arrowhead). E-F) red pulp with congestion, abnormal plasma cell (black arrowhead). E) mast cell (black arrow). F) Abnormal mast cell (white arrowhead), macrophage (arrow)**.**

The microscopic examination revealed that the white pulp was obviously reduced; the red pulp was expanded and packed with lymphocytes and erythrocytes (Chen *et al.,* 2010). Another infection caused by the Goose Nephritic Astro virus showed the destruction of splenic lymphocytes and a starry sky pattern, particularly at the region between the white and red pulp. This pathological injury was reported between the 3rd and 7th-day post-infection (dpi) and improved after the 7th dpi (Ding *et al.,* 2021). The LSB group showed significant enhancements, compared to the HS group, and looked similar to the control group. The low dose of sodium butyrate ameliorated the effect of HS and activated the immune cells and immunity. Several studies have noted that sodium butyrate can improve the avian spleen tissue in variable conditions. For example, spleen germinal center size increased on day 21 and d-35 in broiler chicks treated with 1.0 g/kg sodium butyrate (Sikandar *et al.,* 2017). Likely in the Japanese quail chicks, after being supplemented with SB starting from

hatching, the germinal center sizes/splenic field area were higher at 42 days of life (Elnesr *et al.,* 2019). The microscopic analysis of the spleen in the HSB group revealed an improved microscopic image, even though it remained inferior to the control or LSB groups. In this case, the red pulp has mild congestion and little depletion in the white pulp, with many tangible body macrophages which contain a range of fragments resulting in a "starry sky" pattern. These symptoms resembled several intoxication symptoms mentioned by numerous researchers. For example, the white pulp lymphocytes reduced in high arsenic trioxide (As2 O3)-treated chickens (Zhao *et al.,* 2017). Also, microscopic alterations, such as congestion in the white and red pulp were demonstrated in chickens treated with thiram (Liu *et al.,* 2022). By microscopic examination of the spleen in high molybdenum broilers, there were fewer lymphocytes in the periarterial lymphatic sheath at 21 days old, fewer splenic nodules, and reduced lymphocyte density with a small white pulp and broadened red pulp at ages 36 and 42 days, when compared to those in the control group, which suggested that high Mo in the diet might reduce B-cell and T-cell numbers and disrupt the immune response in broilers (Yang *et al.,* 2011). The lymphocytes in several splenic nodules in selenium-fed broilers were reduced in diverse ways, and the number of splenic nodules was slightly reduced, at 14 days of age. As the dietary selenium level rose, congestion of red pulp also became visible, and hypo cellularity was seen in the splenic corpuscle and periarterial lymphatic sheath at 21 and 28 days of age in the 5, 10, and 15 mg/kg selenium-fed broilers (Peng *et al.,* 2011). Even normal dietary constituents could be harmful at higher doses, such as high-fat diet HFD. In HFD-rats, the majority of the white pulp cells had degenerative alterations, including irregular, dense nuclei, pale and vacuolated cytoplasm, apoptotic bodies, empty large gaps among the cells and deteriorated some central arteriole's wall. The red pulp also showed loss of integrity, and many lymphocytes had heterogeneous or vacuolated cytoplasm and dark uneven nuclei. Numerous cells showed hemosiderin pigment, cytoplasmic accumulation, noticeably swollen blood sinusoids, and blood extravasation. However, rats fed a diet containing high-fat diet + vitamin D revealed that the spleen had restored most of its normal architecture (Mohamed, 2019). The overall histological appearance was better in the HSB group, than the HS group. However, it was still inferior to the LSB group amelioration. Therefore, the best histological architecture was observed in the low-sodium butyrate-treated group.

Exposure to insults such as toxins, chemicals and pathogens has a great adverse effect on immunity. They might inhibit the activity of antioxidant enzymes, which causes free radicals to accumulate in the immune system's organs and cause membrane lipid peroxidation. In chickens treated with arsenic, it is possible to hypothesize that oxidative stress could take place in mitochondria and result in the production of pro-apoptotic proteins into the cytosol, which leads to cellular death (Zhao *et al.,* 2017).

Likely, in HS, the bird's body produces more reactive oxygen species (ROS) to maintain its thermal balance. This causes oxidative stress, which has an adverse effect on oxidation and immunological balance

of immune organs, and further triggers the inflammatory system damaging broiler tissues (Lara & Rostagno, 2013; Hirakawa *et al.,* 2020; Liu *et al.,* 2021; Hu *et al.,* 2022; Barsoum *et al.,* 2019).

In the current study, sodium butyrate stands against the HS and caused histological improvement in the immune organs, such as the thymus and spleen in broiler chickens. According to available literature, butyric acid enhances the lipid metabolism, mineral absorption, and immunological condition of birds by providing antibacterial, anticatabolic, and antioxidant properties. This could be explained by its action on several axes. One of the suggested SB's immune-stimulating mechanisms was by changing immune cell motility, adhesion, cytokine production, and cellular functions like proliferation, activation, and death (Deepa *et al.,* 2018; Liu *et al.,* 2018; Berni Canani *et al.,* 2012). It has been suggested that butyrate increases DNA manufacturing and stimulates growth arrest in the G1 phase of the cell cycle (Liu *et al.,* 2018). The innate immune response, particularly during the early stages of infection, plays a considerably more critical and essential role in host defense. The humoral immunity of broilers is the second line of defense. The butyric acid or its sodium salt might influence the function of B and T lymphocytes. Another role is its anti-inflammatory characteristics via blocking nuclear factor B (NF-kB) activation, inhibiting the manufacturing of interferon-γ, and increasing levels of peroxisome proliferator-activated receptor g (PPARg). This can happen as a response to reducing histone deacetylase (HDAC), butyrate-induced HDAC suppression, and the consequent positive health effects. Many researchers also reported that one of the immune-boosting methods of SB that it reduces the production of nitric oxide, which consequently reduces the expression of pro-inflammatory cytokines like interleukin-1beta (IL-1B), IFN, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) (Sikandar *et al.,* 2017; Zhang *et al.,* 2011; Lan *et al.,* 2020; Ahsan *et al.,* 2016; Zhou *et al.,* 2014; Liu *et al.,* 2018; Berni Canani *et al.,* 2012). There are also suggested effects on pathogens, such as the inhibition of invasion gene expression and reduces bacterial pathogenicity. It is additionally reported to enhance the effectiveness of broiler chickens generally and the qualities of the carcass (Deepa *et al.,* 2018). NF-B is a transcription factor that controls the expression of numerous genes linked to immunology and inflammation, including immunological receptors, adhesion molecules, growth regulators, and pro-inflammatory cytokines and enzymes (Liu *et al.,* 2018). Butyrate, but not acetate or propionate, may stimulate PPAR, which has wide anti-inflammatory impacts in numerous cell types. PPARγ Agonist Peroxisome proliferator-activated receptors (PPARs) are a class of ligand-activated transcription factors stimulated by fatty acids and eicosanoids. It belongs to the nuclear hormone receptor group. PPAR1 is expressed at elevated levels in the large intestine, and immune cells (Yip *et al.,* 2021; Liu *et al.,* 2018).

CONCLUSIONS

Sodium butyrate can be used as a feed additive to protect the spleen from heat stress. The low dose of SB is more effective than the higher dose.

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دراسة مورفولوجية عن الدور المحتمل لزبدات الصوديوم في حماية الطحال في بدارى التسمين المتعرضة للاجهاد الحراري

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يعد الإجهاد الحراري أحد المشكلات الاقتصادية الرئيسية التي تواجه صناعة الدواجن في العالم. إلى جانب انخفاض أداء النمو، يقلل الإجهاد الحراري من مناعة الدجاج أيضًا. الجزء الرئيسي من الوظيفة المناعية هو الجهاز اللمفاوي. الغدة التيموثية وجراب فابريشيوس هما الأعضاء اللمفاوية الرئيسية. أما الطحال فهو العضو اللمفاوي الثانوي. زبدات/بيوتيرات الصوديوم هو ملح الصوديوم لحمض البيوتريك ويقترح استخدامه لتخفيف الإجهاد الحراري. هدفت الدراسة الحالية إلى تقييم تأثير إضافة زبدات الصوديوم على كفاءة الطحال في دجاج التسمين المتعرض للاجهاد الحراري. تم تقسيم ١٥٠ فرخًا سليمًا (عمر يوم واحد) عشوائيًا إلى ٤ مجموعات: ١) المجموعة الظابطة (النظام الغذائي الأساسي ودرجة الحرارة الطبيعية)؛ ٢) مجموعة الإجهاد الحراري (والمعرضة ل 34 درجة مئوية لمدة ٨ ساعات/يوم لمدة ٧ أيام بدءًا من عمر ٢٩ يومًا)؛ ٣) مجموعة بيوتيرات الصوديوم المنخفضة (0.5 جم / كجم كل يوم وتعريض للحرارة العالية و ٤) مجموعة بيوتيرات الصوديوم العالية (2 جم/كجم كل يوم) وتعرض للحرارة العالية. تم الحصول على الطحال في نهاية التجارب (عمر ٣٦ يومًا). كشفت نتائجنا أن HS تسبب في أضرار جسيمة، مثل ضمور ملحوظ وفقدان التنظيم في اللب الأبيض والنزيف في كل من اللب الأبيض واللب الأحمر للطحال. أظهرت الجرعة منخفضة من بيوتيرات الصوديوم حماية واضحة لتركيب اللب النسيجي الصحي للطحال. بيوتيرات الصوديوم وخاصة الجرعة المنخفضة خففت من تأثير الإجهاد الحراري، ولكن أظهرت الجرعة العالية تحسنا أقل في الصورة النسيجية. لذلك نقترح استخدام جرعات منخفضة من بيوتيرات الصوديوم، ونحذر من الجرعات الكبيرة، لمواجهة الإجهاد الحراري في بدارى التسمين.