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Saim Qureshi

Ph.D. Scholar, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-K, Jammu and Kashmir, India

HM Khan

Professor, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-K, Jammu and Kashmir, India

Sana Shafi

Assistant Professor, Department of Environmental Sciences, S.P. College, Cluster University Srinagar, Jammu and Kashmir India

S Adil

Assistant Professor, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-K, Jammu and Kashmir, India

MS Mir

Associate Director Extension, SKUAST-K, Jammu and Kashmir, India

Corresponding Author:

Saim Qureshi

Ph.D. Scholar, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-K, Jammu and Kashmir, India

Effect of cold stress and its various ameliorating remedies on histomorphology of lymphoid glands (Bursa of Fabricius and Spleen) in broiler chicken

Saim Qureshi, HM Khan, Sana Shafi, S Adil and MS Mir

Abstract

The present study was conducted to investigate the effect of cold stress and its various ameliorating measures on histomorphology of lymphoid organs in broiler chicken. 270 day-old commercial broiler chicks were used for the study. The chicks were distributed into 7 treatments of 3 replicates with 13 chicks each. Cold stress was provided at 2 °C to 8 °C for 8 hours from 3rd to 6th week of age in all treatment groups except first and fifth treatment groups. The broiler birds in T₁ and T₅ were reared under normal temperature conditions (25 °C). T₁ was kept as control group. Antioxidant vitamin E 250 mg per kg of feed was supplemented to basal diet in T₃. Chromium 0.1 gram per kg of feed was supplemented to basal diet in T₄. The results revealed that lymphoid glands from T₁ group had no lymphoid depletion and necrosis. T₅ also revealed no lymphoid depletion and necrosis. The lymphoid glands from T₂ showed severe lymphoid depletion and necrosis. The lymphoid glands T₄ displayed moderate lymphoid depletion and necrosis, however, T₇ showed mild lymphoid depletion and necrosis. Mild lymphoid depletion and necrosis was observed in T₃ group. The lymphoid glands from treatment group T₆ revealed mild lymphoid depletion. In conclusion, the cold stress in broiler chicken caused severe pathological changes in lymphoid organs but the cold stress mitigating practices such as early cold conditioning, supplementation of vitamin E and chromium reduced the pathological effects of cold stress in these organs.

Keywords: Cold stress, bursa, spleen, broiler chicken, chromium, Vit E

Introduction

Stress is quite unavoidable to organisms and therefore, its health as well as productivity is always at risk due to stress [25]. There is a certain range of environmental temperature in which broiler chickens maintain a normal body temperature with least involvement of thermoregulatory mechanism. This range of ambient temperature is called a zone of thermoneutrality [6]. The animal is subjected to heat or cold stress when the environmental temperature is beyond the upper or lower limit of the thermoneutral zone [1]. As an environmental stressor, cold stress has adverse effects on the physiology of broiler chickens [3]. The cold stress has negative influence on the productive efficiency including health and disease resistant capacity in broiler chickens [14]. The cold stress has negative impact on the immunity and performance of the broiler chickens by increasing the levels of corticosterone in the blood [12].

Short-term cold conditioning of chickens at an early age causes an improvement in thermotolerance and performance in broiler chickens subjected to cold stress [18] due to epigenetic adaptations [19]. Antioxidants play a pivotal role in the health and nutrition of broiler birds. Vitamin E improves the performance of broiler chickens in cold regions [5] by reducing oxidative stress [11]. Chromium being an essential micro element is very low in poultry ration, because the ingredients used for feed formulation are naturally low in chromium [24]. The requirement of chromium also increases in specific conditions like fatigue, trauma and stress (nutritional, metabolic, physical and environmental) to optimize growth [8]. Various cold stress mitigating measures including early cold conditioning of broiler chicks and use of antioxidants (vitamin E and chromium) as a supplementation in the diet of broiler chickens help to reduce negative effects of cold stress to some extent [14].

Since cold stress influences the immune system of broiler chickens effecting both the humoral and cell mediated immunity [2] and not much literature is available regarding its effect on histomorphology of lymphoid organs, therefore, the present study was conducted to investigate

the effect of cold stress and its various ameliorating measures on the histomorphology of lymphoid organs in broiler chickens.

Material and Methods

Methodology

Two hundred and seventy day-old commercial meat type broiler chicks were procured from a reputed source. The chicks were reared until 14 days of age in battery cages until. During the first seven days period all the birds were provided with a pre-starter mash (23% crude protein). The birds were provided starter (crude protein 22%) and finisher (crude protein 19%) diets from periods first week to third week and fourth week to sixth week of their age respectively. The diets were isonitrogenous, isocaloric and formulated to meet the recommendations of the bureau of Indian standards^[4]. Birds had free access to feed and water throughout and were maintained on a constant 24-hour light schedule. All chicks were vaccinated against Ranikhet disease on 5th day with F1 strain vaccine and IBV-95 vaccine against infectious bursal disease on 16th day. Chicks were checked twice daily for mortality, if any.

Experiment design

During the winter months (December and January) a biological trial was conducted on commercial chicks in the farm of division of Livestock Production and Management, Faculty of Veterinary Sciences at Shuhama, SKUAST-K, Jammu and Kashmir. At third and fourth day of age cold conditioning (2 °C to 8 °C) for 3-4 hours was provided to 78 birds. These early cold conditioned birds were kept separate until distributed into respective treatment groups (fifth and sixth). On fourteenth day (end of second week), the chicks were individually weighed, distributed into seven treatment groups of three replicates with 13 chicks in each in a completely randomized design so that the treatment means differ as little as possible. Cold stress was provided at 2 °C to 8 °C for 8 hours from third week of age to sixth week of their age for all treatment groups except first and fifth treatment groups. The broiler birds in the treatment groups T1 and T5 were reared under normal temperature conditions (25 °C). Treatment group first (T1) was kept as control group. Antioxidant vitamin E 250 mg per kg of feed was supplemented to the basal diet in the third treatment group. Chromium 0.1 gram per kg of feed was supplemented to the basal diet in the fourth treatment group. Chromium 0.2 gram per kg of feed was supplemented to the basal diet in the seventh treatment group. E-Care (Vitamin E) from Gujarat Liqui Pharmacaps India was source of Vitamin E. Chromisac from Zeus Biotech Limited India was source of chromium. The birds were reared on deep litter system throughout the experimental period. The second treatment group was subjected to cold stress and no antioxidant supplementation of any kind was added to the basal diet (Table 1).

Parameters recorded

The tissue samples from Lymphoid glands (Bursa of fabricius and Spleen) were collected from the slaughtered birds (6 birds per treatment) for the histopathological analysis, at the end of experimental period (42 days) and fixed in 10% buffered formalin saline. Tissues were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene, and embedded in paraffin. The casting of blocks was carried out in L-molds

(two L-shaped pieces) which facilitated the manipulation of size as per the requirement. The rotary type microtome was used for cutting the paraffin sections. The blocks were properly trimmed, and the sections of 5 mm thickness were cut. Continuous ribbons (6-7 inches long) of the material were cut and laid on the surface of constant temperature water bath (around 55 °C). The sections were separated with a heated scalpel after they spread completely. The cut sections were mounted on the clean glass slides using Mayer's egg albumin as the section adhesive. The mounted slides were dried in paraffin oven at 60 °C for 1 hour. The tissue sections were stained by the Harris hematoxylin and eosin staining method. The paraffin sections were deparaffinized with the xylene before hydration through graded alcohol to distilled water. This was followed by the dehydration in ascending grades of alcohol. The clearing was performed in the xylene, and a drop of distrene plasticizer xylene mountant was placed on a coverslip and the section on the slide pressed on it. The slide was inverted, and the cover slip was pressed with a rod to remove the air bubbles if any trapped. The prepared slides were observe at a magnification of ×20 under a light microscope fitted with the stage micrometer.

Ethical approval

The study was conducted after approval of research committee and institutional ethical committee (registration no: 1809/GO/ReBi/S/15/CPCSEA).

Results

Histology of bursa of Fabricius and spleen showing the effect of cold stress and its various mitigating remedies in different treatment groups has been presented in Table 2. The lymphoid glands from T₁ group showed no lymphoid depletion and necrosis (Figure 1 & 8). There were no pathological signs. Treatment group T₅ also revealed no lymphoid depletion and necrosis (Figure 5 & 12) on histological examination. Any kind of pathological lesions did not appear. The lymphoid glands from treatment group T₂ showed severe lymphoid depletion and necrosis (Figure 2 & 9). The lymphoid glands T₄ displayed moderate lymphoid depletion and necrosis (Figure 4 & 11). But the treatment group T₇ showed mild lymphoid depletion and necrosis (Figure 7 & 14). Mild lymphoid depletion and necrosis (Figure 3 & 10) was observed on histological examination of lymphoid organs from broiler chickens in the T₃ group. The lymphoid glands from treatment group T₆ revealed mild lymphoid depletion (Figure 6 & 13).

Discussion

The lymphoid glands from treatment group T₅ where early cold conditioning followed rearing under normal temperature conditions and control group (T₁) where broiler chickens were reared under normal temperature showed no lymphoid depletion and necrosis. There were no pathological signs. This may be attributed to normal temperature conditions which do not cause any harmful effects on immune organs^[12, 13].

The lymphoid glands from treatment group where broiler birds were reared under cold challenge and no stress mitigating practices were adopted (T₂) showed severe lymphoid depletion and necrosis. Zikic *et al.*^[23] reported that histological examination of lymphoid organs of broiler chickens under prolonged sound stress revealed progressive reduction in the size of lymphoid follicles, increase of connective tissue and appearance of cysts due to cortisol. Heat

stress causes lymphoid depletion and necrosis with moderate interfollicular fibrosis in lymphoid organs of broiler chickens [16, 20]. Stress is one of the factors responsible for depletion of lymphocytes in the lymphoid organs of broiler chickens [7]. The severe lymphoid depletion in lymphoid organs of broiler chickens reared under cold stress may be attributed to oxidative stress due to cold and immune suppression due to increase in the level of blood cortisol which causes tissue injury [22].

The lymphoid glands from cold stress group supplemented with chromium @ 0.1 g/kg of feed (T₄) displayed moderate lymphoid depletion and necrosis. But the treatment group in which broiler birds under cold stress were supplemented with chromium @ 0.2 g/kg of feed (T₇) showed mild lymphoid depletion and necrosis. T₇ and T₄ groups revealed improvement when compared with T₂ group. Chromium supplementation in broiler chickens under stress influences cytokine production in lymphoid glands which reduces damage due to oxidative stress [15]. There is reduction in the blood cortisol level due to chromium supplementation in the diets of broiler chickens under stress which alleviates immune suppression [17].

Mild lymphoid depletion and necrosis was observed on histological examination of lymphoid organs from cold stressed broiler chickens but supplemented with vitamin E in the diet (T₃). There was seen improvement in T₃ group as

compared with T₂ group. Vitamin E ameliorates aflatoxin induced histopathological alteration in lymphoid organs in broiler chickens [21]. Vitamin E activates glutathione peroxidase and helps in binding of aflatoxin with glutathione-s-transferase as a consequence aflatoxin is removed through urine [21]. Vitamin E reduces pathological changes in lymphoid organs due to stress with significant proliferation of lymphocytes [7]. Kammon *et al.* [9] reported that vitamin E had significant ($p \leq 0.05$) ameliorating effect on immunological alterations induced by imidacloprid chronic toxicity in chickens. The vitamin E supplementation reduces lymphoid depletion, congestion, haemorrhage, oedema and fibrous tissue proliferation in bursa of fabricius and spleen in chickens administered imidacloprid as a toxicant [9]. Histological examination of lymphoid glands of broiler chickens supplemented with vitamin E reveal proliferation of lymphocytes in bursa of fabricius, thymus and caecal tonsil due to its antioxidant activity [10].

The lymphoid glands from treatment group where early cold conditioning of birds followed cold stress (T₆) revealed mild lymphoid depletion. The improvement in the lymphoid glands of T₆ group when compared with T₂ group may be attributed to the reduction in immune suppression due to significant ($p \leq 0.05$) decrease in blood cortisol level and improvement in thyroid and leucocyte function as a result of early cold conditioning of broiler chickens under cold stress [18].

Table 1: Treatments details of experimental plan

Treatment	Details
T ₁	Broiler chickens reared under normal temperature conditions.
T ₂	Broiler chickens reared under cold stress conditions
T ₃	Broiler chickens reared under cold stress and supplemented with vitamin E @ 250 mg/kg of feed
T ₄	Broiler chickens reared under cold stress and supplemented with chromium @ 0.1 g/kg of feed
T ₅	Broiler chickens provided early cold conditioning followed by rearing under normal temperature conditions.
T ₆	Broiler chickens provided early cold conditioning followed by rearing under cold stress conditions.
T ₇	Broiler chickens reared under cold stress and supplemented with chromium @ 0.2 g/kg of feed

Table 2: Level of lymphoid depletion and necrosis in various treatments

Characteristics	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Lymphoid depletion and Necrosis	-	+++	+	++	-	+	+

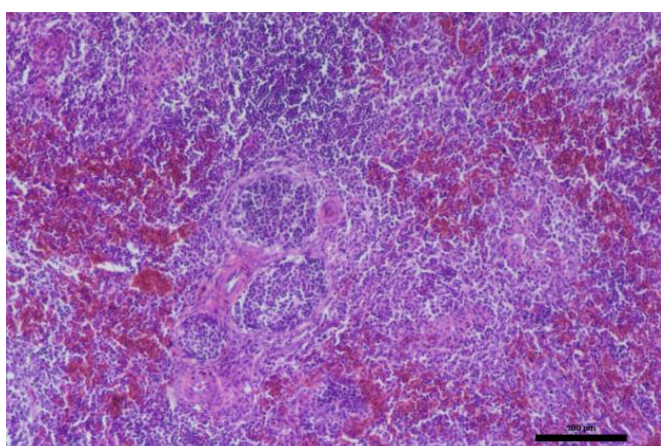


Fig 1: Histological section of Spleen from control group T1 (H&E 20X)

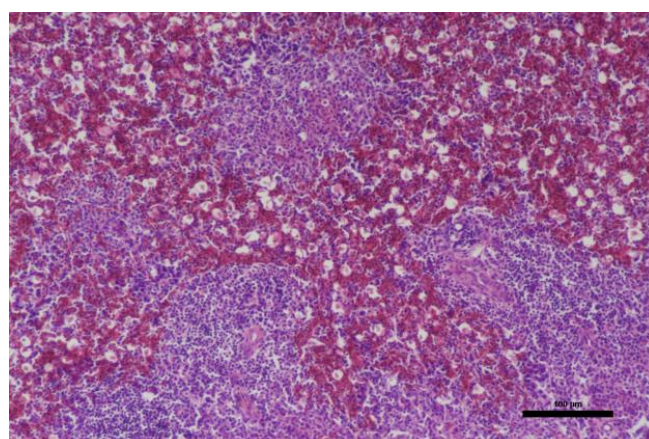


Fig 2: Histological section of Spleen from T2 showing severe lesions of lymphoid depletion and necrosis (H&E 20X)

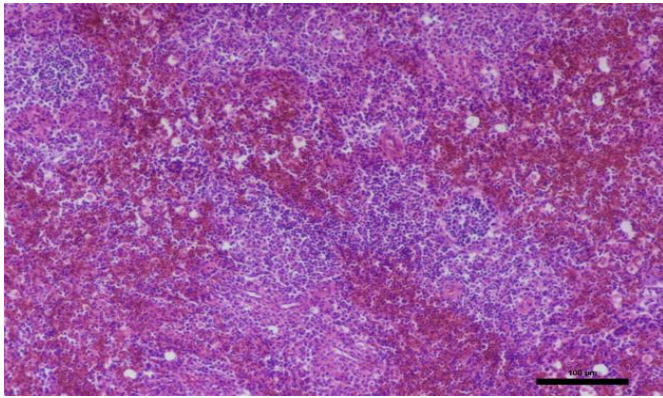


Fig 3: Histological section of Spleen from T3 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

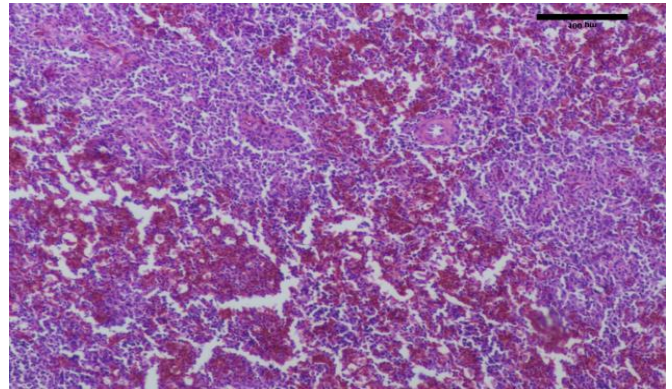


Fig 7: Histological section of Spleen from T7 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

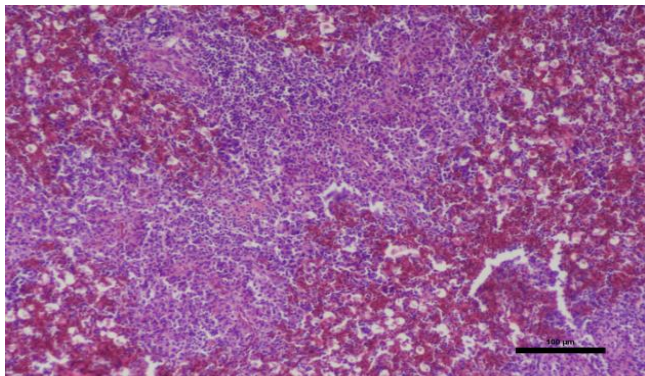


Fig 4: Histological section of Spleen from T4 showing severe to moderate lesions of lymphoid depletion and necrosis (H&E 20X)

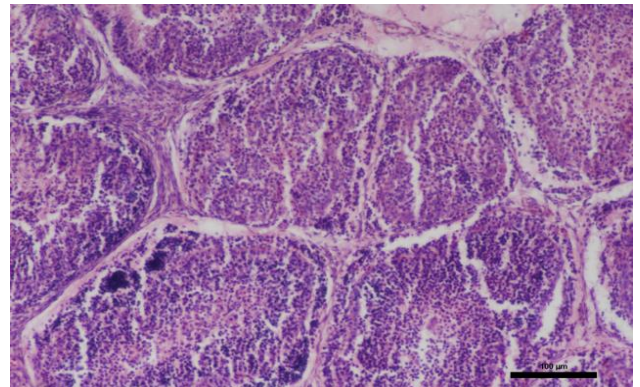


Fig 8: Histological section of Bursa of Fabricius from control group T1 (H&E 20X)

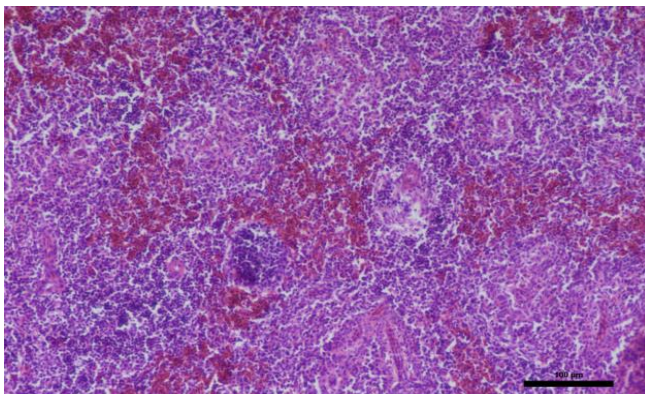


Fig 5: Histological section of Spleen from control group T5 (H&E 20X)

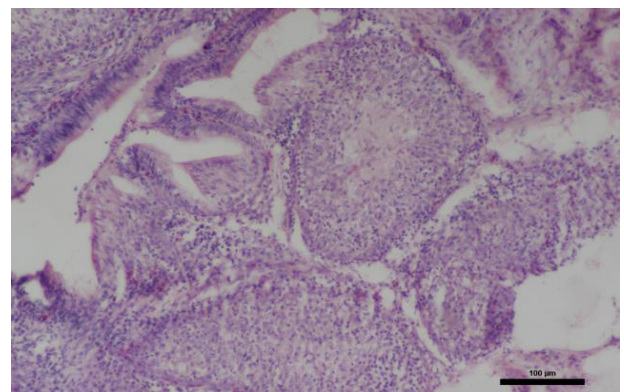


Fig 9: Histological section of Bursa of Fabricius from group T2 showing moderate hypertrophy of cells and distortion of normal architecture with depletion of lipid content (H&E 20X)

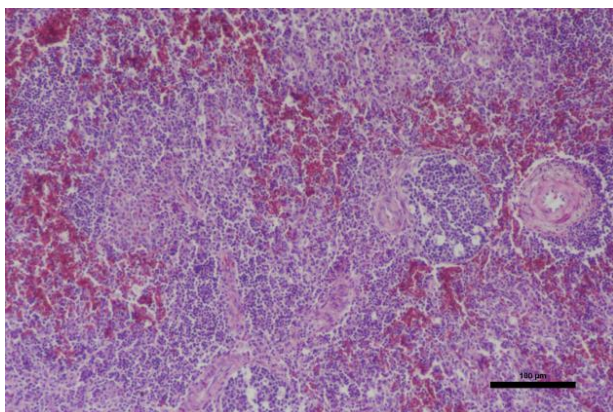


Fig 6: Histological section of Spleen from T6 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

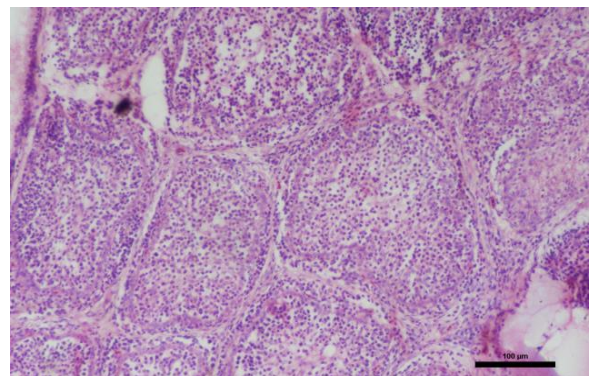


Fig 10: Histological section of Bursa of Fabricius from T3 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

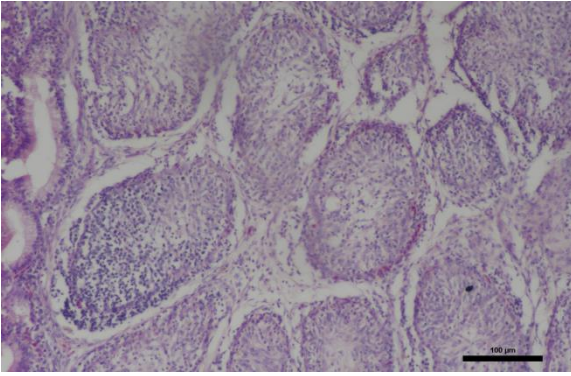


Fig 11: Histological section of Bursa of Fabricius from T4 showing severe to moderate lesions of lymphoid depletion and necrosis (H&E 20X)

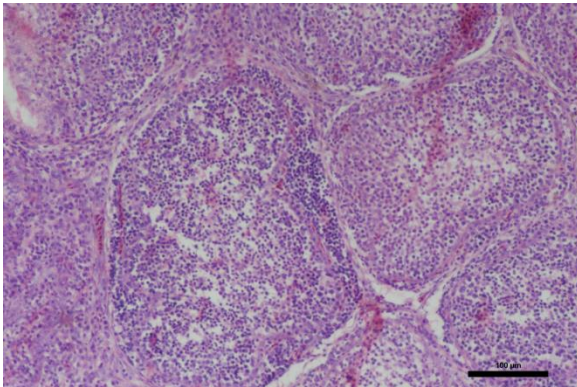


Fig 12: Histological section of Bursa of Fabricius from T5 group (H&E 20X)

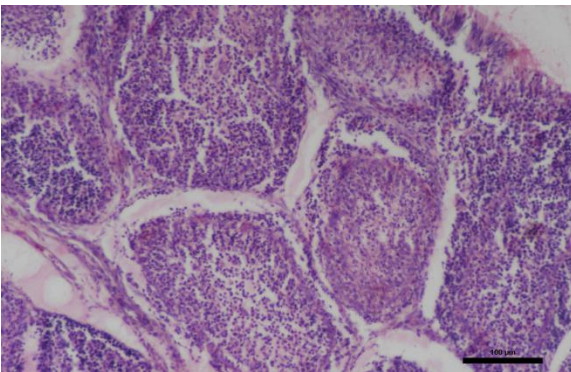


Fig 13: Histological section of Bursa of Fabricius from T6 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

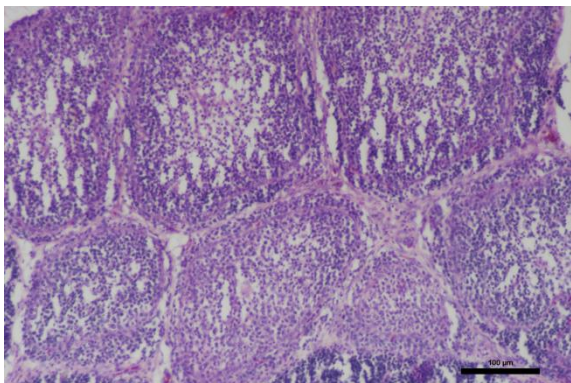


Fig 14: Histological section of Bursa of Fabricius from T7 showing moderate lesions of lymphoid depletion and necrosis (H&E 20X)

Conclusion

The cold stress in broiler chickens caused severe pathological changes in lymphoid organs as was shown in their respective histological sections. But the cold stress mitigating practices such as early cold conditioning, supplementation of vitamin E and chromium reduced the pathological effects of cold stress in these organs. Further, short-term cold conditioning of chickens at an early age resulted in reduction in immune suppression as was revealed by mild depletion of lymphoid organs.

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