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Influence of cold stress and its remedial measures on jejunal Histomorhology of broiler chicken

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Abstract
The environmental stress has been reported to affect gut histomorphology of chicken. Hence, the present study was undertaken to investigate the influence of cold stress and its remedial measures on jejunal histomorphology of broiler chicken. 270 day-old commercial broiler birds were distributed into 7 treatments groups, each having 3 replicates of 13 chicks each. In all treatment groups, birds were subjected to cold stress at 2 0C to 8 0C for 8 hours from 3rd to 6th week of age except T1 and T5 groups. Birds in T1 and T5 were reared under normal temperature conditions (25 0C). T1 served as control group. In T3, antioxidant in the form of vitamin E @ 250 mg per kg of feed was added to basal diet. In T4 and T7, Chromium @ 0.1 gram and 0.2 gram per kg of feed was added to basal diet respectively. Broiler chickens from treatment groups under cold stress (T2, T3, T4, T6 and T7) had significantly ($p \leq 0.05$) lower villus height (VH), crypt depth (CD) and VH/CD ratio with infiltration of inflammatory cells predominantly heterophils in the mucosa when compared with the control group (T1). VH, CD and VH/CD ratio increased numerically but not significantly ($p > 0.05$) in T5 when compared to control. Addition of Vitamin E and Chromium resulted in higher VH, CD and VH/CD ratio when compared to T2. In conclusion, cold stress had adverse effect on jejuna histomorphology, however, cold stress mitigating remedies such as early cold conditioning, supplementation of vitamin E or chromium reduced the pathological effects of cold stress in broiler chicken.

Keywords: chicken, chromium, cold stress, histomorphology, jejunum

Introduction
Poultry sector is one of the rapidly growing industries and contributes significantly towards food security and nutrition. The poultry meat and eggs are consumed globally at a significant proportion. But the poultry production is vulnerable to various types of stresses. The various types of stresses like environmental, nutritional, social and psychological have been reported to reduce bird welfare, performance indices and reproduction activities [1]. There is a range of environmental temperature in broiler birds called as zone of thermoneutrality in which broiler chickens maintain a normal body temperature and beyond the upper or lower limit of this thermoneutral zone, the broiler birds are subjected to stress [2]. The broiler chicks are exposed to cold conditions from time to time because of sudden changes in environmental temperature. Especially, the cold related stress is quite common and a major production hindrance in the northern region of the globe [1, 3]. The cold stress effects the histology of adrenal gland, thereby, changing the corticosteroid level in the blood of broiler birds [4], which in turn is immunosuppressant causing negative impact on lymphoid glands in broiler chickens [5]. The gastrointestinal tract (GIT) is a highly complex and dynamic organ, which plays a vital role in digestion, absorption and immune responses. The oxidative stream is a common manifestation of all stresses [6]. The gastrointestinal tract (GIT) is a highly metabolic active organ as it demands high oxygen supply [7] and therefore is very much susceptible to oxidative stress [6]. The oxidative stress along with increase in the level of corticosteroids in the blood of stressed broiler birds induces intestinal injury, mild enteritis, dysfunction of digestion and absorption, and even compromises intestinal epithelial barrier function resulting in many pathogenic diseases [8]. Inflammation of the intestine also decreases nutrition absorption and consequently decrease in weight gain [6].
Antioxidants like vitamin E or chromium play a very critical role in the poultry nutrition. They help to counter negative effects of stress and therefore need to be supplemented in poultry diet as their synthesis is reduced during stress [1]. Vitamin E supplementation in the diet of broiler chickens is an effective way of alleviating the adverse effects of stress on poultry production [9]. Chromium is an essential micro mineral [10] and its supplementation in the diets of broiler

chickens under stress reduces negative effects of stress by decreasing the corticosteriod level in their blood [11]. Short-term cold conditioning of chicks at an early age causes an improvement in thermotolerance and performance when these broiler chickens were subjected to cold stress in latter part of their lives [1, 12] due to epigenetic adaptations [13]. Therefore, early cold conditioning of broiler chicks can be one of the important remedy to reduce negative effects of cold stress [1, 4, 5].

The environmental stress affects histomorphology [3, 6] and functional dynamism of small intestine especially its jejunum part [8] and not much literature is available regarding the effect of cold stress on the jejunal histomorphology, therefore, the present study was undertaken to investigate the influence of cold stress and its remedial measures on jejunal histomorphology of broiler chicken.

Material and Methods

Methodology

Two hundred and seventy three day-old commercial meat type broiler chicks were procured from a reputed source. The chicks were reared in battery cages until 14 days of age. 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%) diet for second and third week of their age while finisher (crude protein 19%) diet for fourth, fifth and sixth week of their age. The diets were isonitrogenous, isocaloric and formulated to meet the recommendations of the bureau of Indian standards [14]. 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 ^{0}C to 8 ^{0}C) for 3-4 hours was provided to 78 birds. These birds which were early cold conditioned were kept separate until distributed into respective treatment groups (fifth and sixth). At the end of second week (on fourteenth day), the chicks were individually weighed, distributed into seven treatment groups of three replicates, with 13 chicks in each replicate in a completely randomized design with the motive to differ treatment means as little as possible. Cold stress was provided at 2 ^{0}C to 8 ^{0}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 ^{0}C). Treatment group (T1) was kept as control group. In the third treatment group (T3), antioxidant vitamin E @ 250 mg per kg of feed was supplemented to the basal diet. Supplementation of chromium at 0.1 gram per kg of feed was done to the basal diet in the fourth treatment group. Chromium at 0.2 gram per kg of feed was added as a supplement 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 cold stress mitigation measures of any kind were applied to the broiler birds in this group.

Parameters recorded

The tissue samples from Jejunum part of Small Intestine from the slaughtered birds (6 birds per treatment) were collected for the histopathological analysis, at the end of experimental period (42 days). The tissue samples were 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 [15]. 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 (vide: 1809/GO/ReBi/S/15/CPCSEA).

Statistical analysis

The data obtained were statistically assessed by the analysis of variance (ANOVA) through General Linear Model procedure of SPSS (10.0) software considering replicates as experimental units and the values were expressed as means±standard error. Duncan's multiple range test [16] was used to test the significance of difference between means by considering the differences significant at $P< 0.05$.

Results and Discussion

Table 1 shows the histomorphology of jejunum in broiler chickens due to the effect of cold stress and its various mitigating remedies for easy interpretation. The villus height (VH), crypt depth (CD) and Villus height(VH)/Crypt depth (CD) ratio increased numerically but not significantly ($p>0.05$) in treatment group T5 (Figure 5) where early cold conditioned birds were reared under normal temperature when compared with control group (T1) reared under normal temperature (Figure 1). No signs of pathognomic infiltration of inflammatory cells were seen in the mucosa or sub-mucosa of jejunum of broiler chickens reared in the treatment groups

T1 and T5. The treatment groups T1 and T5 showed normal villus architecture in the jejunum. Broiler chickens from treatment groups under cold stress (T2, T3, T4, T6 and T7) had significantly ($p \leq 0.05$) lower villus height, crypt depth and VH/CD ratio with infiltration of inflammatory cells predominantly heterophils in the mucosa when compared with the control group (T1). Among all the cold stress groups villus height, crypt depth and VH/CD ratio were significantly ($p \leq 0.05$) lowest in group reared under cold stress without any cold stress mitigating practice (T2) (Figure 2). There was wide spread and higher infiltration of inflammatory cells, predominantly heterophils in mucosa of jejunum in the treatment group T2 when compared with the treatment groups T3, T4, T6 and T7. The villus height, crypt depth and VH/CD were significantly ($p \leq 0.05$) increased in treatment group T3 (Figure 3) where broiler chickens under cold stress were supplemented with vitamin E when compared with T2 group.

Early cold conditioning improves metabolism so increases growth of cells in the intestine which result in increase in height of villus of intestine [12, 17]. Cold stress in broiler chickens damages mucosa and decreases villus and crypt growth [18]. Heat stress has negative effect on the intestinal mucosa of broiler chickens and produces aberrant changes with reduction in height of villus [19, 20]. The histological examination of jejunum of quails reared under cold stress reveals damage to mucosa and villus. Cold stress alters the Cyclooxygenase-2 (COX-2) and Prostaglandin E synthase expression in the intestine of birds which has negative impact on histomorphology of intestine. COX-2 is pro-inflammator and aberrant COX-2 expression plays a role in the pathogenesis of intestinal inflammation associated with cold stress of longer duration [3]. Prolonged cold stress reduces epithelial cell proliferation rate as well as induces inflammation in the small intestines of rats. The decrease in villus height and crypt depth in broiler chickens reared under cold stress may be attributed to the reduction in proliferation of epithelial cells in the intestine as a result of impaired metabolism due to cold stress [21]. Elevated levels of blood corticosteroids associated with stress in broiler chickens altered intestinal-immune barrier, allowing pathogenic bacteria to migrate through the intestinal mucosa and generating an inflammatory infiltrate. Inflammation of the intestine also decreased nutrition absorption and consequently decreased in weight gain [6].

Previous studies have confirmed that longer intestinal villi indicate an improved ability to absorb nutrients in the intestine [22, 23]. In addition, it has been shown that longer villi are associated with active cell mitosis, which provides a greater absorptive potential of villi for various nutrients [23, 24]. Deeper intestinal villi crypts indicate a rapid metabolism of tissue in order to allow the renewal of the intestinal villi, if there is a need for its regeneration [22, 24]. Lowering the height of the villi or reducing crypt depths of intestinal villi may lead to a reduction in the absorption of nutrients [23, 25]. Murakami *et al.* [26] studied the effect of vitamin E on the histomorphology of mucosa of intestine. They reported better development of the mucosa of the intestine in the broiler chickens supplemented with vitamin E. Vitamin E augments the function of glutathione and prevents peroxidation of cell membrane by free radicals [26]. Newcastle Disease Virus infection causes oxidative stress and histopathological changes in the duodenum and jejunum of broiler chickens which are ameliorated by the supplementation of vitamin E due to its antioxidant property [27]. Antioxidants increase villus height and improve mucosal histomorphology in broiler chickens under heat stress [20].

T6 cold stress group (Figure 6) where broiler birds were given early cold conditioning had significant ($p \leq 0.05$) increase in villus height, crypt depth and VH/CD ratio when compared with T2 group. Early cold conditioning improves thermotolerance and metabolism which increases growth of cells in broiler chickens reared under cold stress [12]. Chromium @ 0.1 g/kg of feed in T4 group (Figure 4) significantly ($p \leq 0.05$) increased villus height, crypt depth and VH/CD ratio when compared with T2 group. Supplementation of chromium @ 0.2g/kg of feed in cold stress group T7 (Figure 7) revealed significantly ($p \leq 0.05$) higher villus height, crypt depth and VH/CD ratio when compared with either T4 or T2 treatment groups. Supplementation of chromium in broiler chickens reared under heat stress significantly ($p \leq 0.05$) increases villus height, crypt depth and VH/CD ratio [28]. The increase in villus height and crypt depth of jejunum due to chromium supplementation may be attributed to its antioxidant activity which reduces cellular damage [10]. There is also improvement of digestion due to chromium supplementation in broiler chickens reared under cold stress [29].

Table 1: Effect of cold stress and its various mitigating remedies on jejunal histology of broiler chickens

	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Villus Height (µm)	1348.31±10.43^e	827.09±7.32^a	1199.57± 981^d	927.48± 5.16^b	1362.07± 8.27^e	1050.11± 6.89^c	1206.37±7.72^d
Crypt depth (µm)	184.70± 5.21^e	137.62±2.85^a	173.60±4.36^d	150.81± 4.16^b	186.33± 5.91^e	164.08± 3.11^c	174.08± 3.67^d
Villus Height/Crypt depth (VH:CD Ratio)	7.30± 0.02^e	6.01± 0.01^a	6.91± 0.03^d	6.15± 0.01^b	7.31±0.03^e	6.40±0.01^c	6.93± 0.02^d

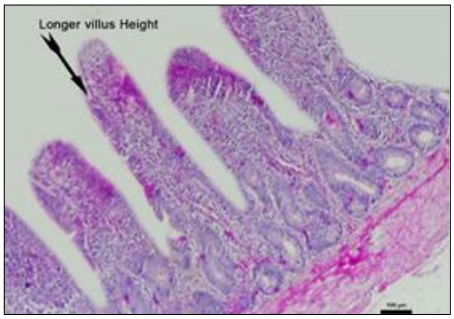

Fig 1: Histological section of Jejunum from control group T1 (H&E 20X)

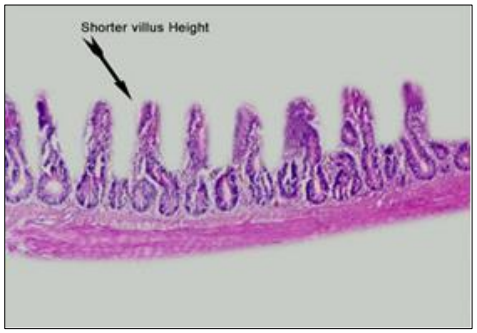

Fig 2: Histological section of Jejunum from T2 group (H&E 20X)

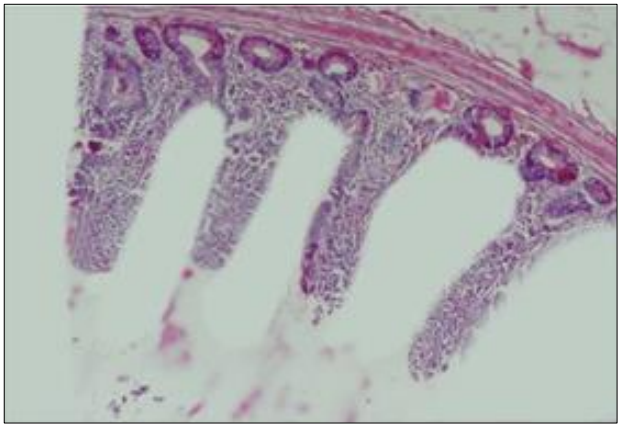

Fig 3: Histological section of Jejunum from T3 group (H&E 20X)

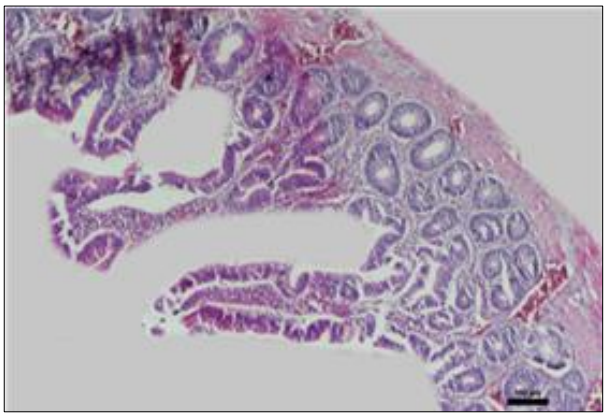

Fig 4: Histological section of Jejunum from T4 group (H&E 20X)

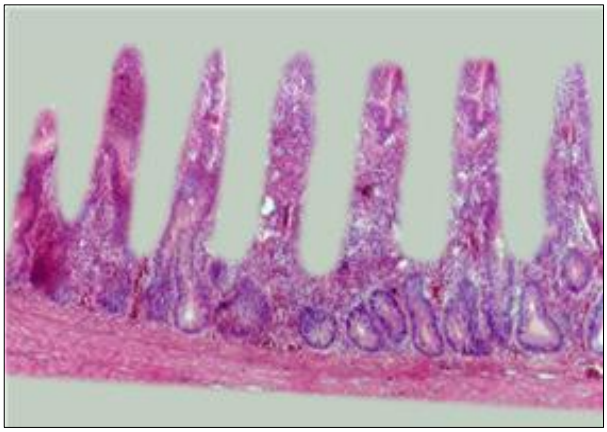

Fig 5: Histological section of Jejunum from T5 group (H&E 20X)

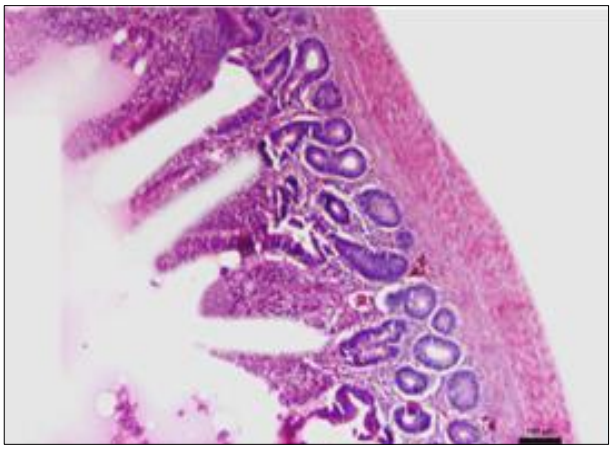

Fig 6: Histological section of Jejunum from T6 group (H&E 20X)

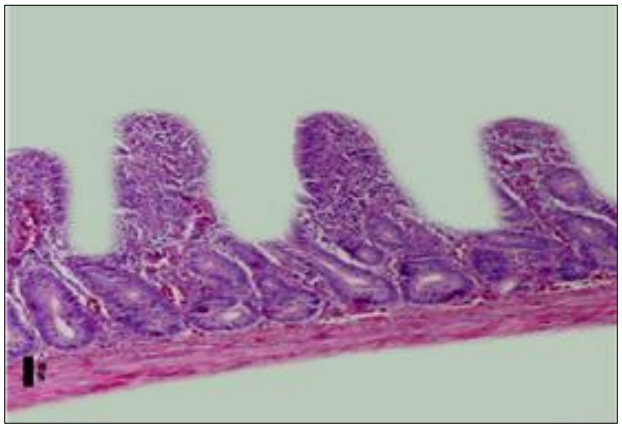

Fig 7: Histological section of Jejunum from T7 group (H&E 20X)

Conclusion

Cold stress had adverse effect on jejuna histomorphology of broiler chicken as depicted by decreased VH, CD and VH/CD ratio along with infiltration of inflammatory cells in the mucosa. However, the cold stress mitigating remedies such as early cold conditioning, supplementation of vitamin E or chromium reduced the pathological effects of cold stress in this part of small intestine.

Conflict of Interest

All authors declare no conflicts of interest.

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