A biological trial was conducted on commercial chicks during the winter months (December and January). Day old commercial meat type broiler chicks (273) were procured from a reputed source. Cold conditioning (2°C to 8°C) at third and fourth day of age 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). At the end of second week, the chicks were individually weighed, distributed into 7 treatment groups of 3 replicates with 13 chicks in each replicate. Cold challenge @ 2°C to 8°C for 8 hours was provided 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 T3 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. The data on individual body weight of the experimental birds and the cumulative feed consumption and feed conversion ratio on group basis were recorded at weekly intervals. Deaths were recorded daily and all dead birds were necropsied to identify ascites syndrome. There was no significant (p<0.05) difference in the average body weight and body weight gain among various treatment groups throughout the experiment period. The cumulative feed consumption showed significant (p<0.05) difference among various treatment groups throughout the experiment period. Highest feed consumption (p<0.05) was observed in broiler chickens reared under cold conditions when compared with broiler birds reared under normal temperature conditions. Among the cold challenge treatment groups (T2, T3, T4, T6 and T7), there was significant (p<0.05) improvement in feed conversion ratio (FCR) in the treatment groups T6 (early cold conditioning birds exposed to cold stress) and T7 (supplementation of chromium 0.2 g/kg of feed to birds exposed to cold stress). Among different treatment groups in general best FCR was observed in treatment group T4 (early cold conditioning group reared under normal conditions) followed by T1 (control group reared under normal conditions). At the end of the biological trial ascites linked mortalities showed significant (p<0.05) difference among various treatment groups. There was no mortality reported in treatment groups kept under normal temperature conditions (T1 and T3). Highest ascites related mortality (23.07%) was observed in treatment group in which cold stress was provided and no measures were taken to alleviate the effect of cold stress on broiler birds (T2). The Vitamin E supplementation in the diet of broiler birds reared under cold stress (T2) showed significant (p<0.05) reduction in ascites related mortality (10.25%).

Keywords: Ascites, Broiler chicken, Early cold conditioning, Chromium, Cold stress, Performance, Vitamin E

INTRODUCTION

The State of Jammu and Kashmir falls in the north-western region of the great Himalayas in India. The average altitude of the valley of Kashmir is 1850 metres above the mean sea level. It is surrounded by mountains, which are always snow-clad. The climate in the valley of Kashmir has its own peculiarities. The season of winter is quite cold which lasts from November to March. These months are characterised by the onset of snow and rain as a consequence of Mediterranean depressions (Raina, 2002). The temperature from December 24 to March 8 is often below zero (Raina, 2002). According to the nineteenth livestock Census report, the total poultry population in the country is 729.2 million and there has been growth in the poultry production by 12.39%. It is estimated that Indian poultry industry contributes about 422 million United States dollars to the GDP (gross domestic production) of the country. But the state of Jammu and Kashmir with only 8 million poultry population, ranks seventh in the Poultry population in the country (Anonymous, 2014).

Thus, the situation in Kashmir regarding the poultry sector is different from the rest of the country. The economy of Kashmir Valley is badly affected due to outflow of the money to outside states owing to the poultry imports into the valley of Kashmir (Gilani, 2009). Our state is also a worse hit when it comes to unemployment and it is assuming enormous proportions with every passing day. The poultry industry is one of the activities which could generate employment. To counter the problem of unemployment we can turn to the self-employment schemes (Banday et al., 2013). This provides us with plenty of avenues to absorb the educated unemployed youth. But there are certain problems related to poultry sector in Kashmir (Gilani, 2009).

Physiological tolerance of organisms is a strong determinant of the environmental conditions in which they inhabit. At certain range of environmental temperature the organisms maintain a normal body temperature with least involvement of thermoregulatory mechanism. This range of ambient temperature is called a zone of thermoneutrality (Kampen et al. 1979). The environmental temperature beyond the upper and lower limit of the thermoneutral zone is supposed to produce heat or cold stress in animals (Meltzer, 1983). The adverse climatic condition produces physiological stress which has profound economic influence on the productive efficiency including health and disease resistant capacity (Phuong et al., 2016). Exposure of poultry birds to extreme temperature stressor modulates the immune responsiveness and haemato-biochemical parameters of birds (Hangalapura et al., 2004). Among all the environmental stressors, cold stress induces physiological responses which are of high priority and energy demanding for homeotherms. Cold temperature can increase ascites susceptibility by increasing both metabolic oxygen requirements and pulmonary hypertension (Stolz et al., 1992). The biggest obstacle in raising broilers at high altitudes and cold conditions is the ascites syndrome. This condition can be characterized by an accumulation of fluid in the abdominal cavity and elevated mortality that tends to peak between 4-6 weeks of age (James, 2005).

In addition to this, the winter rearing of broiler chickens is associated with excess moisture content of the litter material, which in turn results in elevated levels of air contaminants, such as ammonia (Campbell et al., 2008). Chickens can be imbued with better thermal stress tolerance during pre-natal and early post-natal period by epigenetic adaptation mechanisms, characterized as genomic imprinting, which occur to pre-adapt the organism for the expected post-natal environmental conditions (Nichelmann et al., 2001; Nichelmann, 2002; Tzschentke and Basta, 2002). It is based on the influence that environmental conditions may have on the set point of the physiological control systems (Dröner, 1974). It can also be achieved during early post-natal period by thermal conditioning (Arjona et al., 1988 and 1990; Yahav and Hurwitz, 1996; Yahav et al., 1997), or during life span, by acclimation to extreme environmental temperatures (Hurwitz et al., 1980; Yahav et al., 1995). Shinder et al. (2002) reported that short-term cold conditioning of chickens at an early age could induce an improvement either in thermotolerance during cold challenge or in performance of chickens exposed to an optimal environmental temperature.

Antioxidant plays an important role in both nutrition and production performance in poultry. Dietary supplementation of vitamin E at levels of higher than the National Research council (NRC, 1994) recommendations for poultry enhanced the immune response (Lin et al., 2004) and general performance (Guo et al., 2001). The higher doses of vitamin E had positive influence on the productive performance than lower doses in quails (Biswas et al., 2008). It is also suggested that high vitamin E supply can alleviate oxidative stress in Pulmonary Hypertension Syndrome (Iqbal et al., 2002; Niu et al., 2018) and can be beneficial in reducing ascites mortality.
Chromium is an essential micromineral, which is required for nutrient metabolism (Anderson, 1987). Moreover, Chromium content of poultry feed is very low, therefore its requirement increases during stress (Zulfiqar et al., 2016; Mayada et al., 2017). Such circumstances demand for supplementation of this essential trace element to optimize productive performance in poultry (Khan et al., 2014). Based on aforementioned facts, a research study was conducted to evaluate the effect of cold on performance in broiler chicken along with examining effect of early cold conditioning and use of anti-oxidants (Vitamin E and Chromium) on the ability to cope with cold exposure during their life span.

MATERIALS AND METHODS

Methodology
Day-old commercial meat type broiler chicks (273) were procured from a reputed source. 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). They 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 (BIS, 1992). 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
A biological trial was conducted on commercial chicks during the winter months (December and January) in the farm of division of Livestock Production and Management, Faculty of Veterinary Sciences at Shuhama, SKUAST-K. Cold conditioning (2°C to 8°C) at third and fourth day of age 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 challenge 2°C to 8°C for 8 hours was provided 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 T3 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 treatment group second was subjected to cold challenge and no antioxidant supplementation of any kind was added to the basal diet.

Parameter recorded
The data on individual body weight of the experimental birds and the cumulative feed consumption and feed conversion ratio on group basis were recorded at weekly intervals. Deaths were recorded daily and all dead birds were necropsied to identify ascites syndrome.

Ethical approval
The study was conducted after approval of research committee and institutional ethical committee (registration no: 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 (Duncan 1955) was used to test the significance of difference between means by considering the differences significant at p<0.05.

RESULTS AND DISCUSSION
There was no significant (p>0.05) difference in the average body weight and body weight gain among various treatment groups throughout the experiment period (Table 1 and 2). It shows that cold stress did not adversely affect body weight in broiler chicken. The results are in agreement with Blahova et al. (2007). He reported that cold stress did not significantly (p>0.05) effect body weight in broiler chicken. However, Aksit et al. (2008) reported lower body weight gains when broiler birds were subjected to cold stress. But, Leenstra and Cahaner (1991),
in a study investigated genotype and environmental temperature interactions and reported that the low temperature caused the highest growth rate in all genotypes. Actually, body weight of broiler birds reared under cold stress conditions is closely related to their feed consumption.

The cumulative feed consumption showed significant (p<0.05) difference among various treatment groups throughout the experiment period (Table 3). The difference was discernible clearly after third week of their age. At the end of third week lowest feed consumption was recorded in treatment groups reared under normal temperature conditions (T_1 and T_3). The broiler birds reared under cold stress showed significantly (p<0.05) higher feed consumption at the end of third week when compared with the control group. The cumulative feed consumption at the end of sixth week showed similar significant (p<0.05) impact of cold stress on feed consumption and metabolism pattern of broiler chicken (Table 3). Highest cumulative feed consumption (p<0.05) was observed in broiler birds reared under cold conditions when compared with broiler birds reared under normal conditions (Table 3). The results are in concordance with Blahova et al. (2007) and Aksit et al. (2008). They independently reported the increase in feed consumption in broiler chicken reared under cold stress conditions. Poultry are homeotherm animals that can live comfortably only in a relatively narrow zone of thermoneutrality (Blahova et al. 2007). It is in order to balance their body temperatures, birds are forced to increase feed consumption under low temperatures (Aksit et al. 2008). This explains the finding regarding less feed consumption in the treatment groups (T_1 and T_3) as they were not subjected to cold challenge.

| Table 1. Average weekly body weight (kg) of broiler chicken reared under cold conditions (2°C to 8°C for 8 hours) at the farm of faculty of veterinary sciences SKUAST-K in Kashmir region, India |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Week            | T_1             | T_2             | T_3             | T_4             | T_5             | T_6             | T_7             |
| 2               | 368.15±0.27     | 371.99±1.57     | 368.73±4.22     | 370.24±3.11     | 373.68±0.66     | 367.86±3.64     | 369.57±2.27     |
| 3               | 624.83±2.47     | 621.22±5.51     | 625.76±3.68     | 619.88±6.58     | 622.92±7.35     | 627.38±10.32    | 627.98±5.92     |
| 4               | 981.74±5.38     | 978.48±9.26     | 983.11±6.71     | 980.28±2.16     | 984.74±5.38     | 979.53±6.42     | 986.36±7.35     |
| 5               | 1324.91±0.58    | 1329.53±6.18    | 1321.74±3.81    | 1329.67±2.16    | 1319.67±2.16    | 1332.88±2.56    | 1335.18±3.61    |
| 6               | 1708.29±7.65    | 1713.39±1.96    | 1706.87±3.45    | 1714.46±3.98    | 1719.10±3.95    | 1721.62±4.22    | 1718.15±7.24    |

| Table 2. Average body weight gain (kg) of broiler chicken reared under cold conditions (2°C to 8°C for 8 hours) at the farm of faculty of veterinary sciences SKUAST-K in Kashmir region, India |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age in Weeks    | T_1             | T_2             | T_3             | T_4             | T_5             | T_6             | T_7             |
| 2-3             | 256.68±0.79     | 249.23±3.30     | 257.03±2.10     | 249.68±3.66     | 249.24±2.59     | 259.24±1.89     | 258.41±4.98     |
| 2-4             | 613.59±3.63     | 606.49±4.40     | 614.38±8.31     | 611.55±4.45     | 611.06±4.60     | 611.67±8.28     | 616.79±4.11     |
| 2-5             | 956.76±9.46     | 957.54±3.64     | 953.01±3.17     | 959.43±4.95     | 945.67±1.67     | 965.02±2.70     | 965.61±4.49     |
| 2-6             | 1340.14±8.56    | 1341.4±3.89     | 1338.14±4.43    | 1344.22±6.48    | 1345.42±3.27    | 1353.76±5.46    | 1348.58±9.75    |

| Table 3. Average weekly feed consumption (grams) of broiler chicken reared under cold conditions (2°C to 8°C for 8 hours) at the farm of faculty of veterinary sciences SKUAST-K in Kashmir region, India |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age in Weeks    | T_1             | T_2             | T_3             | T_4             | T_5             | T_6             | T_7             |
| 2-3             | 410.68±1.27     | 418.70±3.95     | 431.81±1.51     | 416.96±2.85     | 398.78±0.85     | 433.39±1.96     | 431.54±102      |
| 2-4             | 1135.14±2.95    | 1225.11±1.97    | 1241.04±1.85    | 1229.21±3.35    | 1124.35±3.05    | 1211.10±1.23    | 1215.07±0.85    |
| 2-5             | 1923.08±2.26    | 2135.31±2.50    | 2115.68±1.63    | 2129.93±2.03    | 1881.88±2.25    | 2074.79±2.35    | 2085.71±1.29    |
| 2-6             | 3082.02±0.85    | 3407.15±3.67    | 3385.49±2.26    | 3400.87±1.25    | 3054.10±3.5     | 3357.32±0.95    | 3344.47±1.38    |

Means within the same row with different superscripts are significantly different (p<0.05)
They suggested that a diet containing chromium (Shinder et al., 2002) could be considered as a protective practice in poultry to lessen the depressive effects of cold stress to certain extend if not completely. The beneficial impacts of chromium have been linked with improvement in the functions of liver and pancreas (Blahova et al., 2003; Yardimci et al., 2006) and epigenetic adaptation mechanisms, characterized as genomic imprinting, which occur to pre-adapt the organism for the expected post-natal environmental conditions (Nichelmann et al., 2001; Nichelmann, 2002; Tzschentke and Basta, 2002).

The beneficial effect of chromium in alleviating the effect of cold stress in poultry was also reported by Sahin and Sahin (2001). They suggested that a diet containing chromium can be considered as a protective practice in poultry to lessen the depressive effects of cold stress to certain extend if not completely. The beneficial impacts of chromium have been linked with improvement in the metabolism and immune system in the poultry (Mayada et al., 2017). Dietary supplementation of chromium stimulate the secretion of digestive enzymes by improving the functions of liver and pancreas (Sahin et al., 2005; Onderci et al., 2005; Toghyani et al., 2010; Noori et al.,

Table 4. Average weekly feed conversion ratio of broiler chicken reared under cold conditions (2°C to 8°C for 8 hours) at the farm of faculty of veterinary sciences SKUAST-K in Kashmir region, India

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Treatment Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>2-3</td>
<td>1.6±0.03a</td>
</tr>
<tr>
<td>2-4</td>
<td>1.85±0.01a</td>
</tr>
<tr>
<td>2-5</td>
<td>2.01±0.05a</td>
</tr>
<tr>
<td>2-6</td>
<td>2.3±0.08b</td>
</tr>
</tbody>
</table>

Means within the same row with different superscripts are significantly different (p≤0.05)

Table 5. Related mortality percentage of ascites in broiler chicken reared under cold conditions (2°C to 8°C for 8 hours) at the farm of faculty of veterinary sciences SKUAST-K in Kashmir region, India

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mortality percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0± 0.0a</td>
</tr>
<tr>
<td>T2</td>
<td>23.07± 4.43d</td>
</tr>
<tr>
<td>T3</td>
<td>10.25± 2.51b</td>
</tr>
<tr>
<td>T4</td>
<td>20.51± 256c</td>
</tr>
<tr>
<td>T5</td>
<td>0±0.0a</td>
</tr>
<tr>
<td>T6</td>
<td>15.38± 0.0c</td>
</tr>
<tr>
<td>T7</td>
<td>15.38± 4.43c</td>
</tr>
</tbody>
</table>

Means within the same row with different superscripts are significantly different (p≤0.05)
The ascites related mortality rate during the experiment are given per treatment group in Table 5. At the end of the biological trial ascites linked mortalities showed significant (p<0.05) difference among various treatment groups. There was no mortality reported in treatment groups kept under normal temperature conditions (T1 and T3). Highest ascites related mortality percentage (23.07%) was observed in treatment group in which cold stress was provided and no measures were taken to alleviate the effect of cold stress on broiler birds (T2). Cold temperature increase ascites susceptibility by increasing both metabolic oxygen requirements and pulmonary hypertension (Stolz et al., 1992). Lowest ascites related mortality was reported in treatment group (T3) in which broiler birds kept under cold stress were supplemented with vitamin E 250 mg/ kg of feed. In these birds, dietary vitamin E supplementation could not entirely prevent ascites mortality induced by cold stress but caused significant (p<0.05) decrease to 10.25%. The result is in agreement with Aksit et al. (2008) who reported vitamin E significantly (p<0.05) decreased ascites related mortalities in broiler birds exposed to cold stress. Bottje et al. (1995) have shown that vitamin E reduced ascites-induced mortality probably by providing an increase in antioxidant defence against free radicals (Niu et al., 2018).

The mortality percentage in the treatment group in which early cold conditioning before broiler birds were subjected to cold stress was done (T6) and the treatment group (T7) in which supplementation of chromium 0.2 g per kg of feed was given to broiler birds kept under cold stress was equal (15.38%). The mortality percentage was significantly (p<0.05) lower when compared with the treatment group T2. The early cold conditioning significantly (p<0.05) decreased ascites related mortalities has been reported by other workers also (Shinder et al., 2002; Bahadoran and Hassanzadeh 2009). Schinder et al. (2007) reported that early cold conditioning increased the ability of broiler birds to maintain body temperature and thermotolerance during second cold challenge in later part of their life which in turn decreased incidence of ascites related mortality. This could be related to the change in the endogenous functions of chickens, such as the levels of plasma corticosterone and thyroid hormones. The change of these important parameters is important to epigenetic adoptions that might be beneficial to the metabolic rate or the structural size of the cardiopulmonary systems in broiler chicken (Bahadoran and Hassanzadeh, 2009).

The stress increased production of free radicals which damages the body cells and result in increased poultry mortality and chromium is able to reduce stress due to its antioxidant property which in turn reduces the mortality (Mayada et al., 2017). As cold stress exacerbate a marginal chromium deficiency or increase in requirement, thus implying chromium should be supplemented in the diets of broiler chicken reared under cold stress (Sahin and Sahin, 2002).

CONCLUSION

It can be very well put forward that the temperature of environment is one of the most significant abiotic factors that can influence metabolism and subsequently the production of broiler chickens to the great extent. But various remedies such as providing early cold conditioning to chicks, supplementing vitamin E 250 mg per kg of feed and Chromium 0.2 g per kg of feed can help reduce cold stress.

DECLARATIONS

Acknowledgements
The authors are highly grateful to Head Division of LPM, SKUAST-K for financial support during the study.

Competing interests
The authors declare that they have no competing interests.

Author’s contributions
All the authors have made substantive contribution to the study.

Consent to publish
All the authors gave their informed consent prior to their inclusion in the study.

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