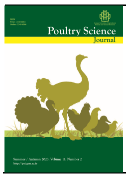


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# Impact of Dietary Inclusion of Organic Zinc and Chromium on Physiological Response of Broiler Chickens Exposed to Cold Stress

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**Abstract**

The study was planned on 120 one-day-old broiler chickens, randomly distributed into 5 dietary treatments with 3 replicates having eight chickens each. Control birds (CON) were reared under thermoneutral conditions and offered a basal diet to meet their nutrient requirements. Treatment CS was offered the basal diet under cold stress. While, treatments $ZNC_{100}$, $CR_{2,}$ and ZN+CR were offered basal diet under cold stress, supplemented with organic Zinc, organic Chromium or their combinations at 100, 2, or 100 + 2 mg/kg DM, respectively. Birds under CS treatment had higher feed intake compared to other treatments. Body weight gain and feed conversion ratio were not significantly affected by treatments. Digestibility of dry matter, crude protein, and nitrogen-free extra of birds under CON treatment were greater than CS, $ZNC_{100}$, and $CR_2$ treatments. The concentration of blood urea nitrogen and albumin to globulin ratio in CS treatment was higher compared to CON treatment. The antibody response of 21-day-old-broiler chickens in the CS group was lower than the birds of the ZN+CR group. The lowest activities of glutathione peroxidase and superoxide dismutase were found in the blood serum of the CS group. The percentage of dressing carcass in both CON and CS treatments were lower than those of birds under ZN+CR treatment. Moreover, the yield of breast and drumstick of the ZN+CR treatment was higher ($P < 0.05$) than the CON treatment. There was better production performance along with improved immune status in broiler birds fed a diet supplemented with organic Zinc and Chromium alone or their combination under cold stress.

## Introduction

In homeeotherms among all the environmental stressors, cold stress is high priority and energy-demanding which reults in oxidative stress (Qureshi *et al.*, 2018) which leads to tissue injury, poor health with low economic returns (Panda and Cherian, 2014). Broiler chicken production is only profitable when maintained at the thermoneutral zone (i.e., 18-25 ºC), as temperature declines below this range, cold stress is induced (Olanrewaju *et al.*, 2010). Supplementation of dietry antioxidants like minerals, vitamins, herbal extracts and certain chemicals are crucial to overcome enivronmental cols stress and pathogens (Maggini *et al.*, 2007; Tomlinson *et al.*, 2008). To combat oxidative stress caused by environmental stressors, trace minerals such as Selenium, Zinc, Chromium, Copper, and Manganese are recommended as external antioxidants (Willcox *et al.*, 2004) and deficiency of these elements causes oxidative stress, thereby affecting the performance of broiler birds with significant mortality. Because of low absorption of inorganic trace minerals, these minerals are administered double to ten times in poultry diets to their NRC requirements resulting in higher excretion of these minerals in poultry litter and ultimately causing either toxicity or deficiency (Aksu *et al.*, 2010). Contrary to inorganic minerals, organic trace minerals (such as Zinc and Chromium) are more bioavailable, resulting in reduced dietary inclusion, hence causing less environmental pollution (Nollet *et al.*, 2007; Zhao *et al.*, 2010). In addition, the organic form of trace minerals possesses different absorption

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pathways through the intestinal wall and do not interfere with other minerals (Mateos *et al*., 2005) resulting in better feed conversion and body weight gain in broilers (Abdallah *et al*., 2009; Das *et al*., 2014). Therefore, the better performance with dietary supplementation of organic trace mineral is possibly due to their better bioavailability (Burrell *et al*., 2004), enzyme-activating properties (MacDonald, 2000) ,and better bone mineralization (Bruerton, 2005).

Among the trace minerals, Zinc has a significant role against oxidative injury stem from environmental stressors by maintaining biological membrane integrity (Bray and Bettger, 1990). There are about two hundred zinc-dependent enzymes in the body to play a significant role in the biochemical pathways (Sharma and Joshi, 2005; Hosnedlova *et al*., 2007). Zinc plays an important role in protein biosynthesis as being an essential component of DNA and RNA polymerase enzymes (Underwood and Suttle, 2001). Also, it plays a vital role in antibody and cell-mediated immune response (Shankar and Prasad, 1998). Similarly, Chromium, an essential trace mineral, improves metabolism and immune system in poultry, hence optimizing productive performance and aiding in reducing stress due to its anti-oxidant property so decreasing the incidence of mortality in poultry (Qureshi *et al*., 2018; Sahin and Sahin, 2001; Mayada *et al*., 2017). Supplementation of Chromium also improves the function of the liver and pancreas and stimulates the secretion of digestive enzymes (Sahin *et al*., 2005; Onderci *et al*., 2005; Toghyani *et al*., 2010; Noori *et al*., 2012; Ebrahimzadeh *et al*., 2013; Hesham *et al*., 2014; Haq *et al*., 2017). So, dietary supplementation of Chromium is essential in broiler birds reared under cold climatic conditions (Sahin and Sahin, 2002). Based on the above facts, the study was framed on the efficacy of organic Zinc and Chromium on broiler performance under cold stress conditions. This is first of its kind study in India where organic trace minerals were used in poultry under cold stress conditions.

## Material and Methods

### Experimental design

During the experiment, the birds were handled as per approved trial protocol regulations of the Institutional Animal Ethics Committee of SKUAST-Kashmir (Reg. no.: 1809/GO/ReL/15/CPCSEA) vide order number AU/FVS/PS-57/3373; dated: 05-07-2021. In the experiment of 5 weeks, 120 one-day-old commercial Venkys Cobb 430Y broiler chicks of either sex were procured from the hatchery and brooded in the battery brooder at 32-35 ºC for the first week. On the 8th day, the chicks were distributed randomly into five treatments, having three replicates with eight chickens each. The basal diet (Table 1) was formulated as per the requirements given by ICAR (2013) (Table 1). Out of five treatments, CON represented positive control (offered basal diet, without cold stress), whereas birds of the CS group received a basal diet under cold stress and served as a negative control. Birds of $ZNC_{100}$, $CR_2$, and ZN+CR treatments received basal diet under cold stress, supplemented with organic zinc at 100 mg/kg diet, organic chromium at 2 mg/kg feed on a DM basis, and a combination of the organic zinc and organic chromium dosages, respectively. Birds of all treatments were reared under similar managemental conditions like space, light, and ventilation, but the CON group was provided a thermoneutral environment (27.28-27.85°C) with the help of room heating equipment, and the remaining treatment groups (CS, $ZNC_{100}$, $CR_2$, and ZN+CR) were reared under cold stress condition (9.99-14.50°C). A week before the commencement of the experiment, all the birds were vaccinated against Marek's disease post-hatching,Infectious bronchitis, Infectious bursal disease, and New Castle's disease. Experimental shed was fumigated and battery brooders were cleaned and disinfected prior to experiment. Cleaned drinking water was offered *ad-libitum* throughout the experiment and mortality if any was recorded. Feed ingredients and complete diets formulated used in the study were analyzed for the proximate constituents (AOAC, 2005), cell wall constituents (Van Soest *et al*., 1991), and minerals (Ca, P, Cu, and Zn) using atomic absorption spectrophotometer (GBC SensAA, GBC Scientific Equipment, Inc., Australia). Different performance parameters like periodical body weight, average daily feed intake, and feed conversion ratio were monitored during the experiment. For determination of nutrient digestibility, a metabolic trial of seven days after allowing a 3-day preliminary period was conducted in the last week of the experiment. For the biochemical analysis of blood serum, glucose was estimated by glucometer instantly after blood collection, other biochemical parameters (Total protein, albumin, blood urea nitrogen, and creatinine) and serum enzymes (Aspartate aminotransferase, alanine aminotransferase, glutathione peroxidase, superoxide dismutase, lipid peroxidase, and catalase) were estimated using commercial diagnostic kits (Span diagnostics limited, Surat, Gujarat, India).

six birds from each treatment were slaughtered for carcass study and samples from breast and thigh portions were taken for oxidative stress parameter estimation. Carcass parameters like percent breast yield, back yield, thigh yield, drumstick yield, neck yield, wing yield, and giblet yield were measured and results were expressed as percent organ weight (g). The ileum, cecal tonsils, bursa of Fabricius and spleen were weighed individually.

**Table 1.** The percentage of ingredients and nutrient composition of the experimental diet (Dry matter basis)

| Ingredients | Diet | | |
|---|---|---|---|
| | starter (1-14 days) | Grower (15-28 days) | Finisher (29-45 days) |
| Yellow maize (CP=9.30%) | 45.85 | 43.74 | 48.34 |
| Soybean meal (CP=45.23%) | 37.40 | 29.06 | 23.73 |
| Wheat bran | 8.00 | 18.84 | 20.00 |
| Soyabean oil | 4.64 | 4.50 | 4.34 |
| Limestone powder | 1.70 | 1.74 | 1.59 |
| Dicalcium phosphate | 1.20 | 1.00 | 0.90 |
| Common Salt | 0.29 | 0.29 | 0.29 |
| Vitamin-mineral premix* | 0.75 | 0.75 | 0.75 |
| DL-Methionine | 0.17 | 0.08 | 0.06 |
| Chemical analysis | | | |
| Metabolizable Energy (Kcal/kg) | 3,096 | 3,141 | 3,181 |
| Dry matter (%) | 92.00 | 92.76 | 90.22 |
| Crude protein (%) | 23.00 | 20.26 | 18.20 |
| Crude fiber (%) | 2.43 | 3.12 | 4.18 |
| Ether extract (%) | 11.09 | 10.88 | 8.56 |
| Ash (%) | 4.09 | 4.34 | 4.76 |
| Methionine (%) | 0.45 | 0.40 | 0.38 |
| Methionine + Cystine (%) | 1.00 | 0.90 | 0.81 |
| Calcium (%) | 1.00 | 0.98 | 0.89 |
| Available phosphorus (%) | 0.30 | 0.28 | 0.25 |

*Supplied per kilogram of diet: vitamin A, 8800 IU; vitamin $D_3$, 3300 IU; vitamin E, 1100 IU; Riboflavin, 9mg; Biotin, 0.25mg; Thiamine, 4mg; Pantothenic acid, 11mg; vitamin $B_{12}$, 13µg; Niacin, 26mg; Choline, 900mg; Vitamin K, 1.5mg; Folic acid, 1.5mg; Ethoxyquin,125mg; Manganese, 55mg as manganous oxide 60%; zinc,50mg as zinc oxide 72%; copper, 5mg as copper sulfate 25%; iron, 30mg as ferrous sulfate 30% and selenium, 0.1mg.

To monitor the immune response of birds, haemagglutination test against the Newcastle disease vaccine was done on the 21st and 28th day after vaccination by β-method (diluted serum and constant virus). The activity of different oxidative enzymes like superoxide dismutase and glutathione peroxidase in erythrocytes was determined by the method of Marklund and Marklund (1974) and Hafeman *et al.* (1974) respectively. Lipid peroxidation activity in erythrocytes and activity of serum catalase was determined as per protocol by Shafiq-u-Rehman (1984) and Aebi (1983), respectively. Erythrocytes membrane peroxidative damage was determined by thiobarbituric acid in terms of malondialdehyde production.

**Statistical analysis of data**

The data generated throughout the experiment were analyzed by one-way analysis of variance using the SPSS software package (Ver. 20.0 for Windows, SPSS, Inc., Chicago, IL). The level of significance among the mean values was tested using Duncan's Multiple Range Test with a significant level of difference set as $P < 0.05$.

**Results**

The results of the overall performance and nutrient digestibility of broiler chicken subjected to organic mineral supplementation under cold stress have been presented in Table 2. Total feed intake of CS treatment was highest when compared to the birds of others groups kept in cold stress. Moreover, Birds that received $ZN_{100}$ diet showed higher feed intake than those of $CR_2$ and ZN+CR treatments. Digestibility of DM and NFE of CON and CS treatments were respectively the highest and the lowest compared to other treatments. Digestibility of CP in CON and ZN+CR treatments was significantly greater than in other groups. Also, the lowest CP digestibility was related to the CS treatment.

The total protein and globulin concentration of serum in the birds of the CS group were significantly lower ($P < 0.05$) than birds received other experimental groups (Table 3). Furthermore, the use of ZN+CR treatment significantly increased total protein and globulin concentration in blood serum compared to other groups (except for $CR_2$). There was a significantly higher serum albumin to globulin ratio recorded in birds of the CS group than those of the CON groups. The concentration of blood urea nitrogen in the CS group was higher than in other treatments. The use of $ZNC_{100}$ and $CR_2$ increased the concentration of blood urea nitrogen compared to CON and ZN+CR groups.

**Table 2.** Total body weight gain, feed intake, feed conversion ratio and nutrient digestibility of broiler birds subjected to organic mineral supplementation

| Parameters [1] | Treatments[2] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | CON | CS | $ZNC_{100}$ | $CR_2$ | ZN+CR | |
| IBW (g) | 181.13 ± 2.28 | 178.33 ± 1.09 | 183.96±2.44 | 179.00 ± 1.49 | 184.13 ±1.64 | 0.07 |
| FBW (g) | 1421.96 ± 16.82 | 1361.75 ±19.22 | 1441.79 ±11.74 | 1424.42 ±15.00 | 1463.63±17.95 | 0.10 |
| TWG (g) | 1240.83 ±17.11 | 1183.42 ±19.44 | 1257.83 ±11.74 | 1245.42 ±15.42 | 1279.50 ±17.95 | 0.05 |
| TFI (g) | 1800.02 ±31.23$^{bc}$ | 2062.57 ±46.53$^{a}$ | 1896.77 ±54.41$^{b}$ | 1701.69 ±33.34$^{cd}$ | 1646.07 ±14.94$^{d}$ | 0.01 |
| FCR (g/g) | 1.45 ±0.03$^{b}$ | 1.74 ±0.04$^{a}$ | 1.46 ±0.08$^{b}$ | 1.39 ±0.05$^{b}$ | 1.29 ±0.01$^{b}$ | 0.05 |
| Nutrient digestibility [3] (%) | | | | | | |
| DM | 69.66 ±0.52$^{a}$ | 59.88 ±1.31$^{c}$ | 66.20 ±0.98$^{b}$ | 65.61 ±0.44$^{b}$ | 67.05 ±0.33$^{b}$ | 0.03 |
| CP | 58.74 ±0.63$^{a}$ | 43.38 ±1.60$^{c}$ | 52.94 ±1.10$^{b}$ | 51.18 ±0.71$^{b}$ | 56.40 ±0.86$^{a}$ | 0.04 |
| EE | 66.74 ±0.50 | 55.88 ±1.20 | 60.81 ±0.87 | 57.11 ±0.66 | 64.01±1.76 | 0.05 |
| CF | 44.57 ±0.85 | 35.08 ±1.86 | 40.66 ±1.46 | 44.22 ±0.78 | 46.08 ±0.44 | 0.05 |
| NFE | 62.81 ±0.56$^{a}$ | 57.37 ±1.15$^{c}$ | 60.01 ±0.90$^{b}$ | 60.49 ±0.64$^{b}$ | 59.69 ±0.32$^{b}$ | 0.03 |

$^{a-c}$ Means within different superscripts in the same row differ significantly ($P < 0.05$).

[1] IBW= Initial body weight; FBW= Final body weight; TWG= Total feed intake; TFI= Total feed intake; FCR=Feed conversion ratio.

[2] Control (CON) - thermoneutral group; CS - Cold stress without supplementation, $ZNC_{100}$- Cold stress, a basal diet supplemented with Zn-Lysine at 100 mg/kg DM; $CR_2$ -Cold stress, a basal diet supplemented with Cr-Propionate at 2 mg/kg DM and ZN+CR- Cold stress, a basal diet supplemented with Zn-Lysine + Cr-Propionate at 100 mg and 2 mg/kg DM, respectively.

[3] DM= Dry matter; CP=Crude protein; EE=Ether extract; CF=Crude fibre; NFE=Nitrogen free extract

**Table 3.** Average serum biochemical parameters in broiler chicken subjected to organic mineral supplementation.

| Biochemical Parameters | Treatment[1] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | CON | CS | $ZNC_{100}$ | $CR_2$ | ZN+CR | |
| Total protein (g/dL) | 2.41±0.40$^{b}$ | 1.94 ±0.17$^{c}$ | 2.46 ±0.32$^{b}$ | 2.19 ±0.33$^{ab}$ | 3.29 ±0.50$^{a}$ | 0.01 |
| Albumin (g/dL) | 0.73 ±0.07 | 0.73 ±0.08 | 0.95 ±0.11 | 0.84 ±0.14 | 1.18 ±0.16 | 0.05 |
| Globulin (g/L) | 1.69 ±0.37$^{b}$ | 0.76 ±0.16$^{c}$ | 1.51 ±0.27$^{b}$ | 1.34 ±0.28$^{b}$ | 2.56 ±0.49$^{a}$ | 0.01 |
| Albumin: Globulin | 0.70 ±0.19$^{b}$ | 1.69 ±0.52$^{a}$ | 0.97±0.32$^{ab}$ | 1.01 ±0.22$^{ab}$ | 0.97 ±0.46$^{ab}$ | 0.04 |
| BUN [2](mg/dL) | 0.37 ±0.07$^{d}$ | 0.96 ±0.07$^{a}$ | 0.56 ±0.01$^{b}$ | 0.55 ±0.02$^{b}$ | 0.48 ±0.08$^{c}$ | 0.03 |
| Creatinine (mg/dL) | 0.15 ±0.01 | 0.16 ±0.03 | 0.20 ±0.02 | 0.14 ±0.03 | 0.15 ±0.02 | 0.39 |
| Glucose (mg/dL) | 390.78 ±15.23 | 347.00±5.35 | 347.22 ±12. 24 | 303.00 ±1.05 | 302.33 ±3.88 | 0.05 |
| Enzyme Activity [3] (U/L) | | | | | | |
| AST | 121.31 ±21.99 | 145.03 ±17.70 | 148.21 ±5.06 | 135.67 ±13.18 | 104.89 ±11.78 | 0.24 |
| ALT | 10.99 ±1.57 | 12.21 ±1.84 | 13.77 ±1.80 | 8.08 ±1.91 | 9.11 ±2.29 | 0.23 |

$^{a-c}$ Means within different superscripts in the same row differ significantly ($P < 0.05$).

[1] Control (CON) - thermoneutral group; CS - Cold stress without supplementation, $ZNC_{100}$- Cold stress, a basal diet supplemented with Zn-Lysine at 100 mg/kg DM; $CR_2$ -Cold stress, a basal diet supplemented with Cr-Propionate at 2 mg/kg DM and ZN+CR- Cold stress, a basal diet supplemented with Zn-Lysine + Cr-Propionate at 100 mg and 2 mg/kg DM, respectively.

[2] BUN=Blood urea nitrogen.

[3] AST= Aspartate aminotransferase; ALT= Alanine aminotransferase

Humoral immune response and immune organ weight in broiler chicken subjected to organic mineral supplementation are depicted in Table 4. On the 21st day, the birds of the CS group produced a lower ($P < 0.05$) antibody response than the birds of the ZN+CR group. But as the age of the birds progressed, on the 28th day, the birds of the CS and $ZNC_{100}$ groups produced lower ($P < 0.05$) antibody responses compared to the birds of the CON group. There was no significant difference in the weights of the immune organs and ileum among all treatment groups.

Results of dietary treatments on serum oxidative stress parameters of broiler chickens were shown in Table 5. The lowest activity of glutathione peroxidase and superoxide dismutase was observed in CS treatment. Lipid peroxidase activity was higher in the birds of the CS group than in birds of the CON and ZN+CR groups (Table 5). However, serum catalase activity was not affected by treatments.

The carcass and cutability parameters of broiler chicken subjected to organic mineral supplementation are presented in Table 6. Higher dressing percentage and bleeding loss were reported in birds of ZN+CR groups when compared to CON and CS groups. Moreover, the dressing percentage of ZN+CR treatment was greater than the $ZNC_{100}$ group. The lowest feathering loss was found in the CON group. The birds of the ZN+CR group had higher ($P < 0.05$) breast and back percentages than CON and CS groups. The percentage of back in the ZN+CR group was greater than in the $CR_2$ group. The percentage of drumstick and thigh in the ZN+CR group was significantly greater when compared to CON and CS, respectively.

**Table 4.** Humoral immune response (against Newcastle vaccine) and immune organ weight in broiler chicken subjected to organic mineral supplementation

| Age (days) | Treatment[1] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | CON | CS | $ZNC_{100}$ | $CR_2$ | ZN+CR | |
| 21 | 56.89 ±25.31$^{ab}$ | 39.11 ±11.3$^{b}$ | 96.00 ±13.06$^{ab}$ | 78.22 ±22.70$^{ab}$ | 106.67 ±21.33$^{a}$ | 0.02 |
| 28 | 156.44 ±18.81$^{a}$ | 78.22 ±9.41$^{b}$ | 64.00 ±0.00$^{b}$ | 135.11 ±51.65$^{ab}$ | 128.00 ± 0.00$^{ab}$ | 0.01 |
| Immune organs (g) | | | | | | |
| Spleen | 1.41 ±0.09 | 1.38 ±0.13 | 1.53 ±0.19 | 1.38 ±0.13 | 1.60 ±0.09 | 0.28 |
| Bursa | 1.80 ±0.27 | 1.07 ±0.12 | 1.35 ±0.13 | 1.10 ±0.13 | 1.44 ±0.14 | 0.05 |
| Caecal tonsils | 0.61 ±0.09 | 0.53 ±0.06 | 0.59 ±0.05 | 0.54 ±0.06 | 0.59 ±0.07 | 0.44 |
| Ileum | 11.35 ±0.47 | 11.04 ±0.25 | 11.70 ±0.54 | 11.68 ±0.40 | 12.00 ±0.42 | 0.16 |

$^{a\text{-}c}$ Means within different superscripts in the same row differ significantly ($P < 0.05$).

[1] Control (CON) - thermoneutral group; CS - Cold stress without supplementation, $ZNC_{100}$- Cold stress, a basal diet supplemented with Zn-Lysine at 100 mg/kg DM; $CR_2$ -Cold stress, a basal diet supplemented with Cr-Propionate at 2 mg/kg DM and ZN+CR- Cold stress, a basal diet supplemented with Zn-Lysine + Cr-Propionate at 100 mg and 2 mg/kg DM, respectively.

**Table 5.** Average serum oxidative stress parameters in broiler chicken subjected to organic mineral supplementation

| Oxidative stress enzymes (IU/mg protein) | Treatment[1] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | CON | CS | $ZNC_{100}$ | $CR_2$ | ZN+CR | |
| Glutathione Peroxidase | 37.77 ±10.07$^{a}$ | 21.07 ±5.22$^{b}$ | 35.61 ±7.18$^{a}$ | 40.60 ±11.14$^{a}$ | 42.97 ±8.08$^{a}$ | <0.005 |
| Superoxide dismutase | 5.73 ±0.12$^{a}$ | 3.58 ±0.19$^{b}$ | 5.71 ±0.09$^{a}$ | 5.79 ±0.13$^{a}$ | 5.80 ±0.13$^{a}$ | 0.04 |
| Lipid peroxidase | 0.70 ±0.02$^{b}$ | 0.83 ±0.01$^{a}$ | 0.75 ±0.19$^{ab}$ | 0.76 ±0.15$^{ab}$ | 0.70 ±0.16$^{b}$ | 0.04 |
| Catalase | 24.01 ±3.21 | 23.13 ±2.88 | 22.56 ±3.76 | 24.08 ±3.43 | 23.22 ±1.97 | 0.06 |

$^{a\text{-}c}$ Means within different superscripts in the same row differ significantly ($P < 0.05$).

[1] Control (CON) - thermoneutral group; CS - Cold stress without supplementation, $ZNC_{100}$- Cold stress, a basal diet supplemented with Zn-Lysine at 100 mg/kg DM; $CR_2$ -Cold stress, a basal diet supplemented with Cr-Propionate at 2 mg/kg DM and ZN+CR- Cold stress, a basal diet supplemented with Zn-Lysine + Cr-Propionate at 100 mg and 2 mg/kg DM, respectively.

**Table 6.** Carcass and percent cutability parameters of broiler chicken subjected to organic mineral supplementation

| Carcass parameters (%) | Treatment[1] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | CON | CS | $ZNC_{100}$ | $CR_2$ | ZN+CR | |
| Dressing carcass | 73.31 ±1.24$^{c}$ | 71.40 ±1.64$^{c}$ | 76.84 ±0.45$^{bc}$ | 74.82±0.92$^{ab}$ | 79.17 ±0.28$^{a}$ | 0.001 |
| Feathering loss | 6.65 ±0.29$^{b}$ | 8.77 ±0.39$^{a}$ | 8.08 ±0.37$^{a}$ | 8.06 ±0.18$^{a}$ | 8.18 ±0.09$^{a}$ | 0.009 |
| Bleeding loss | 3.66 ±0.13$^{b}$ | 3.58 ±0.35$^{b}$ | 4.39 ±0.34$^{ab}$ | 4.14 ±0.38$^{ab}$ | 4.96 ±0.11$^{a}$ | 0.007 |
| Cutability Parameters (%) | | | | | | |
| Breast | 24.14 ±0.77$^{b}$ | 24.36 ±0.48$^{b}$ | 25.19 ±0.46$^{ab}$ | 25.52 ±0.18$^{ab}$ | 27.17 ±0.83$^{a}$ | 0.008 |
| Back | 9.38 ±0.18$^{c}$ | 10.00 ±0.37$^{c}$ | 11.08 ±0.32$^{ab}$ | 10.10 ±0.23$^{bc}$ | 11.23 ±0.18$^{a}$ | 0.003 |
| Drumstick | 10.70 ±0.28$^{b}$ | 9.70 ±0.32$^{ab}$ | 10.69 ±0.31$^{ab}$ | 10.68 ±0.46$^{ab}$ | 11.14 ±0.38$^{a}$ | 0.02 |
| Thighs | 11.52 ±0.58$^{ab}$ | 10.84 ±0.23$^{b}$ | 12.62 ±0.33$^{a}$ | 11.49 ±0.41$^{ab}$ | 12.25 ±0.27$^{a}$ | 0.02 |
| Wings | 9.61 ±0.23 | 9.93 ±0.36 | 10.52 ±0.37 | 10.15 ±0.24 | 10.39 ±0.25 | 0.63 |
| Neck | 5.86 ±0.17 | 6.06 ±0.44 | 6.36 ±0.44 | 6.33 ±0.38 | 6.80 ±0.27 | 0.06 |
| Total Giblet | 5.33 ±0.22 | 5.45 ±0.24 | 5.56 ±0.13 | 5.25 ±0.22 | 5.94 ±0.26 | 0.28 |

$^{a\text{-}c}$ Means within different superscripts in the same row differ significantly ($P < 0.05$).

[1] Control (CON) - thermoneutral group; CS - Cold stress without supplementation, $ZNC_{100}$- Cold stress, a basal diet supplemented with Zn-Lysine at 100 mg/kg DM; $CR_2$ -Cold stress, a basal diet supplemented with Cr-Propionate at 2 mg/kg DM and ZN+CR- Cold stress, a basal diet supplemented with Zn-Lysine + Cr-Propionate at 100 mg and 2 mg/kg DM, respectively.

## Discussion

Environmental stressors, particularly cold stress, are responsible for the induction of physiological oxidative stress (Phuong *et al*., 2016) in broiler chicken, leading to a profound consequence on its productivity and economic efficiency.Better growth performance in broiler chicken is achived under thermoneutral temperature (18-25 ºC) supplemented with antioxidants like traceminerals, vitamins nad herbs to prevent oxidative stress (Olanrewaju *et al*., 2010). Trace minerals such as Zinc, Copper, Chromium, Manganese, and Selenium have been recommended as external antioxidants to manage oxidative stress caused by environmental stressors (Willcox *et al*., 2004). Organic trace minerals like Zinc and Chromium are more bioavailable, resulting in reduced dietary inclusion with reduced fecal excretion, hence causing less environmental pollution (Nollet *et al*., 2007; Petrovic *et al*., 2010; Zhao *et al*., 2010) compared to inorganic minerals. Our research supplemented organic Zinc and Chromium to assess the performance parameters of broilers under cold stress.

### Performance parameters

The total feed intake and feed conversion ratio was significantly ($P < 0.05$) higher in birds kept under cold stress without organic mineral supplementation as compared to birds of thermo neutral group and organic mineral supplemented groups kept in cold stress. The higher feed intake and feed conversion ratio in birds under cold stress without supplementation is possibly due to lower nutrient digestibility and higher gastrointestinal motility under stress. These results are in agreement with the findings that Zinc and Chromium supplementation has a positive influence on the performance of birds by improving feed intake, nutrient utilization, and growth in broiler birds (Qureshi *et al*., 2018; Das *et al*., 2014; Burrell *et al*., 2004; Sahin and Sahin, 2001; Bird, 1995; Salahuddin *et al*., 2000; Lagana *et al*., 2007; Blahova *et al*., 2007; Akşit *et al*., 2008). Besides the positive effects of organic Zinc and Chromium supplementation, mortality pattern was recorded at about 16.66% in Zinc supplemented group (4th and 5th week), 4.16% in Zinc+Chromium supplemented group (4th week), and no mortality was recorded in Chromium supplemented group ($T_3$). The reason for death is the development of ascites observed during post-mortem analysis. It has been found that a higher growth rate in broilers makes them more susceptible to ascites (Debski *et al*., 2004). Also, higher altitudes resulting in reduced partial pressure of oxygen and a high incidence of ascites in broiler birds (Preuss *et al*., 1997; Ma *et al*., 2011). Chromium being the potent antioxidant, successfully helped control mortality in broiler birds by increasing resistance to stress conditions (Sahin and Sahin, 2002; Akşit *et al*., 2008).

### Nutrient digestibility

The present study revealed better nutrient digestibility in the thermoneutral group, and organic mineral-supplemented groups compared to the cold stress group without any supplementation. This is supported by the fact that lower ambient temperatures suppress nutrient digestibility in laying hens, and when birds are supplemented with Zn and Cr, the digestibility of different nutrients is maintained since these minerals have a protective role on pancreatic tissues against oxidative damage (Alves *et al*., 2012). Additionally, Zinc and Chromium supplementation improve gut histomorphology, causing an increase in intestinal maturity and improving the digestibility of nutrients (Onderci *et al*., 2005; Yalcinkaya *et al*., 2012; Tawfeek *et al*., 2014).

### Biochemical parameters

The present finding goes along with Daneshyar *et al.* (2009), implying that lower total protein was reported in the blood of cold-temperature-treated birds than in the normal temperature-treated birds. The serum biochemical parameters such as serum total protein, albumin, and globulin were not influenced significantly due to the interaction of dietary Zn and Cr in the diets. However, Suri (2012) reported that serum total protein concentration was significantly ($P < 0.05$) higher in layers supplemented with 70 mg organic zinc compared to the dietary treatments with inorganic Zinc or organic Zinc at higher or lower doses. BUN and creatinine are the two major nitrogenous wastes found in blood and removed via urine. With increase in BUN and creatinine there is reduced glomeluar filitration due to inadequate renal perfusionThe increased BUN in birds of the CS group may be because of impaired kidney function. Huang *et al.* (2007) reported that serum results in broiler chickens showed that the level of serum BUN significantly increased due to stress (heat stress).

The glucose metabolism of the birds needs to be increased to boost thermoregulation in high-energy demanding situations like cold stress. The CON group showed significantly higher ($P < 0.05$) blood glucose levels than the birds under cold stress. Heat or cold stress increases circulating concentrations of corticosterone in broilers, and it is well-documented that corticosterone reduces insulin sensitivity in broilers (Zhao *et al.*, 2010). The present study witnessed lower blood glucose levels in the groups supplemented with chromium, indicating better glucose utilization in the body. In accordance with the present findings, Sahin and Sahin, (2001) reported a linear increase ($P < 0.001$) in plasma insulin concentration on chromium supplementation under cold stress, which results in increased tissue uptake and utilization of glucose in the body, thus causing a reduction in blood glucose level. There have been reports of decreased ($P < 0.05$) serum glucose concentration before heat stress due to dietary supplementation of Cr (Karami *et al.*, 2018; Tawfeek *et al.*, 2014; Trivedi *et al.*, 2020), which are in accordance with our experimental results. Similar to these findings, the organic Zn-supplemented birds revealed significantly higher blood glucose levels than the organic Cr-supplemented birds in consistence with the reports proposed by Yalcinkaya *et al.* (2012).

The activity of AST and ALT is an indicator of damage to the liver and muscles (McGill, 2016). However, both AST and ALT activities in the present study did not show any adverse effect of supplementation of organic chromium as a feed additive in the diets of broilers. Similarly, Trivedi *et al.* (2020) reported that organic chromium as a feed additive in broilers' diets does not cause the AST and ALT activities to change adversely.

**Immune parameters**

**Humoral Immune parameters**

The birds reared under cold stress without supplementation and zinc-supplemented birds produced a significantly ($P < 0.05$) lower antibody response than the birds supplemented with both Zinc and chromium in combination under cold stress. The present study is in agreement with the reports of Ghazi *et al.* (2012) and Patel *et al.* (2019) who reported that birds supplemented with organic Cr had better antibody responses. Eze *et al.* (2014) also reported that total antibody titers to Newcastle Disease vaccines were much higher in the group of chicks that received chromium propionate in their diet.

**Immune organ weight**

There was no significant difference observed in the average weights of the spleen, caecal tonsils, and ileum among all treatment groups. However, a significantly ($P < 0.05$) lower mean weight of bursa was observed in birds reared under cold stress without mineral supplementation and chromium supplemented compared to those birds reared under thermoneutral temperature. Rama Rao *et al.* (2012) also reported that the relative mass of lymphoid organs (bursa, spleen, and thymus) was not affected by organic mineral supplementation in a broiler diet.

**Serum Oxidative stress enzymes**

The production of ‘reactive oxygen species (ROS) or free radicals is directly related to the rate of metabolism, which results in the occurrence of oxidative damage to tissues, the development of chronic diseases, and a compromised immune system (Rahman *et al.*, 2014). Significantly ($P < 0.05$) lower plasma glutathione peroxidase and superoxide dismutase with higher lipid peroxidase activities were observed in the birds under cold stress compared to birds of the thermoneutral group. In accordance with the present findings, De Grande *et al.* (2020) reported improved oxidative status in broilers supplemented with zinc amino acid complex. Similarly, Ivanišinová *et al.* (2016) and Saleh *et al*., (2018) reported that intake of zinc-proteinate and zinc-methionine significantly increased superoxide dismutase (SOD) activity ($P < 0.05$) in erythrocytes and decreased lipid peroxidation ($P < 0.01$) in plasma of broiler birds. Tawfeek *et al.* (2014) also reported that Cr supplementation at 0.5 mg/kg diet significantly decreased serum malondialdehyde (lipid peroxidation) and increased serum glutathione peroxidase. Also, Onderci *et al.* (2005) reported that chromium and Zinc can be used in the diet of laying hens for alleviating the detrimental effects of cold stress. Suri (2012) also reported significantly ($P < 0.05$) higher superoxide dismutase activity with zinc supplementation, without affecting catalase activity in broiler birds.

**Carcass parameters**

In carcass parameters, significantly higher dressing percentage and bleeding loss were recorded in Zinc and Chromium supplemented groups, and possibly due to their higher body weights. These results are in close agreement with the studies of (Alves *et al*., 2012; Grashorn and Clostermann, 2002; Castellini *et al*., 2002), who reported that birds with higher growth potential are also heavier at slaughter with higher carcass weights. Additionally, the higher cutability percentage notably higher breast, drumstick, back, and thigh percentages in Zinc supplemented and Zn+Cr supplemented groups were recorded compared to other groups, and it is due to higher body weight in these groups, and this finding corroborates with the findings of (Narinç *et al*., 2015; Albuquerque *et al*., 2003; Nikolova and Pavlovskim, 2009). Similarly, Jahanian *et al*., (2008) reported that organic zinc increased breast meat yield in broiler chicken with no effect on other carcass parameters. Some authors, however, reported limited advantages to Zn fortification above established requirements on carcass parameters (Rama Rao *et al*., 2016; Albuquerque *et al*., 2003; Nikolova and Pavlovskim, 2009). Regarding supplementation of Chromium (Trivedi *et al.*, 2020) reported that dietary supplementation of organic chromium propionate had no significant ($P > 0.05$) effect on carcass traits, dressing percentage, and weights of liver, heart, gizzard, kidney, and spleen in broiler chickens.

**Conclusion and future directions**

During cold stress, when broiler birds were supplemented with organic Zinc and Chromium alone or in combination, better production parameters were recorded along with increased profitability. In comparison to the organic Cr-supplemented diet, the use of organic Zinc alone, though, had a better growth performance, feed efficiency, nutrient digestibility, and carcass parameters. Still, due to the higher mortality rate in organic Zinc supplemented birds, these achievements were not economical.

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**Conflict of interest**

All authors declare no conflict of interest.

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