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Alleviation of cold stress induced oxidative stress in White Leghorn Chicken by Chromium and Vitamin C supplementation

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Mizoram being located at an altitude of 1020 m and 23°44'12N latitude enjoys a pleasant climate throughout the year except for a very short period of winter towards the end of December to January. As perceived from the local farmers, poultry farming in winter in Mizoram suffers from great loss due to cold associated diseases and complications.

Environmental stress was found to cause vitamin C and chromium deficiencies in poultry (Sahin and Sahin 2001) and the requirements of dietary chromium and vitamin C during thermal stress have been indicated in poultry (Mowat 1994). Stress marker, heat shock protein (HSP70) has been indicated to associate cold stress in broiler and indigenous chicken (Chen *et al.* 2014, Zhao *et al.* 2014). In addition, thermal stress contributes to oxidative stress in a living organism as a result of more production of reactive oxygen metabolites (Sies 1997). Antioxidant enzyme, superoxide dismutase (SOD) is produced to protect cellular damage during oxidative stress (Altan *et al.* 2003, Imtiaz *et al.* 2014). SOD and HSP70 expression have been reported to be associated with the degree of thermo-tolerance of cattle (Mayengbam *et al.* 2015) with variations in seasons (Mayengbam *et al*. 2017).

Some research findings indicated beneficial impacts of supplementation of chromium and vitamin C on certain egg characteristics of chicken during cold stress (Sahin and Sahin 2001). The present study was therefore carried out to evaluate the impact of supplementary chromium and vitamin C on cold stress induced antioxidative status and certain egg characteristics of White Leghorn chicken during winter.

A total of apparently healthy 60 White Leghorn layer chicken in the age of 42 weeks were reared in Poultry Unit, Instructional Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Mizoram from 1st December 2019 to 29th January, 2020. The birds were divided into 4 groups, viz. control group (C), chromium picolinate (Himedia) supplementation group (CR), vitamin C (ascorbic acid, Himedia) supplementation group (VC) and chromium picolinate (Himedia) and vitamin C (Himedia) supplementation group (CRVC). Male to female sex ratio of 1:7 was maintained in each group. Feed supplements were provided as per the details presented in Table 1. The birds were given their basal diet @120 g/bird/day with an *ad lib.* supply of drinking water. Feeding was done two times a day. Per cent composition of feed provided to the birds is presented in Table 1.

Table 1. Feeds details

Group	Feed
C	Basal diet
CR	Basal diet + Chromium picolinate @400 µg/kg of feed
VC	Basal diet + Vitamin C @250 mg/kg of feed
CRVC	Basal diet + Chromium picolinate @400 µg/kg of feed +Vitamin C @250 mg/kg of feed

Composition of feed in the basal diet

Ingredient	Concentration (%)
Maize	64.80
Soybean meal	26.20
Fish meal	3.50
Rice bran oil	2.21
Dicalcium phosphate	1.25
Sodium chloride	0.30
Limestone powder	1.15
Methionine	0.27
L-Lysine	0.12
L-Threonine	0.045
Toxin binder	0.067
Trace mineral P	0.067
Vitamin premix	0.017
Choline chloride	0.067
Antioxidant	0.01

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Meteorological data, viz. ambient temperature (AT) and relative humidity (RH) were recorded using Watch Dog Automatic Weather Station. AT and RH were recorded at 0, 6, 12 and 18 h of the day at 4 different periods from 1st December, 2019 to 29th January, 2020. The different periods were P1: 1-15 December, 2019; P2: 16-30 December, 2019; P3: 31 December, 2019-14 January, 2020 and P4: 15-29 January, 2020.

Blood samples were collected by venipuncture of wing veins by using 24-gauge needle with syringe on 0, 30th and 60th day of experiment. Blood was collected in Na-heparin coated vials. Plasma was immediately separated by centrifugation of whole blood at 1500 rpm for 1 h. HSP70 and SOD were estimated in plasma using enzyme linked immunoassay (ELISA) kits from Biocodon Technologies, USA as per the prescribed procedures.

Ten eggs were collected irrespective of size and weight from every group of birds at the interval of 15 days i.e. 0, 15th, 30th, 45th, 60th and 75th day. Egg weight, egg length and egg width were recorded immediately after collection of eggs. Egg weight (g) was measured individually by using a digital weighing balance. Egg length (mm) and egg width were measured individually using a Vernier calliper.

Data were analysed by using the statistical software SPSS 16.0 to find out the effects of feed supplements on plasma HSP70, SOD, egg weight, egg length and egg width of White Leghorn chicken. One way ANOVA was applied to find out the variation of means of different groups on different days of treatment. Independent t-test was applied to find out the variation of means between the groups on different days of egg collection.

The lowest average AT with lowest minimum AT was recorded at 6 h in all the periods. Both lowest average AT and lowest minimum AT were recorded in P4 (Supplementary Table 1). The highest average RH with highest maximum RH was recorded at 6 h during P3 (Supplementary Table 2). The optimal AT and RH for poultry rearing was indicated to be 15 to 20°C and 60-70% respectively (Kocaman *et al.* 2005). Yahav *et al.* (1998) reported that RH plays a major role in body weight gain and food intake in growing turkeys with deterioration of turkeys when RH was above 70%. The present finding of lower AT and higher RH during the study indicated that the laying hens had experienced cold stress with high RH.

HSPs are intracellular proteins whose expression was markedly increased by heat shock (Lindquist 1986). Both acute and chronic cold stresses influenced the expression of HSP70 in broiler chicken (Zhao *et al.* 2014) and other species (Prava and Upadhyay 2014, Prava *et al.* 2015). The estimate of HSP70 of different groups in the present study on 0, 30th and 60th day of treatment indicated that HSP70 of group C on 60th day was significantly higher than that on 0 day (Table 2). On the other hand, HSP70 estimated on 60th day in groups CR, VC and CRVC was significantly lower than that estimated on 0 day of treatment. Increase in HSP70 during winter indicated presence of cold stress in layers, as acute cold exposure caused increase in expression of HSP70 in Huainan Partridge chicken (Chen *et al.* 2014). In group CR, the HSP70 remained stable till 30th day of treatment and decreased significantly on 60th day of treatment. In group VC, HSP70 estimated on 60th day was significantly lower than that recorded on 0 day while there were no significant difference between 0 day and 30th day of treatment and between 30th day and 60th day of treatment. HSP70 of group CRVC showed significant decrease from 0 day to 30th day and further to 60th day of treatment. Drop in HSP70 in feed supplemented groups indicated alleviation of cold stress by supplementation of chromium picolinate, vitamin C and their combinations in White Leghorn chicken. In previous studies, dietary supplementation of vitamin C was found to lower heat stress induced HSP70 in broilers (Mahmoud *et al.* 2003).

Table 2. HSP70 (ng/ml) and SOD (pg/ml) of White Leghorn chicken with or without supplementation of chromium and vitamin C

Day	Group C	Group CR	Group VC	Group CRVC
		HSP70 (ng/ml)		
0	10.86±0.55^{a}	15.57±0.36^{b}	14.70±0.69^{b}	15.60±1.18^{c}
30	12.00±0.69^{ab}	14.13±0.48^{b}	13.42±0.64^{ab}	11.84±0.86^{b}
60	13.79±0.53^{b}	11.00±0.71^{a}	11.94±1.11^{a}	8.30±0.28^{a}
P-value	0.011**	<0.001**	0.048*	<0.001**
		SOD (pg/ml)		
0	1.47±0.09^{a}	4.27±0.30^{c}	5.76±0.41^{c}	2.00±0.20^{a}
30	4.66±0.36^{b}	1.75±0.10^{a}	3.17±0.42^{b}	4.02±0.42^{b}
60	6.75±1.03^{c}	2.70±0.09^{b}	1.78±0.21^{a}	2.52±0.16^{a}
P-value	<0.001**	<0.001**	<0.001**	<0.001**

Means in the same column with different superscripts differ significantly.

Heat and cold stress had been found to increase SOD in different species of animals (Ramnath and Rekha 2011, Yatoo *et al.* 2014, Mayengbam *et al.* 2015, Yadav *et al.* 2015, Maibam *et al*. 2017, Mayengbam *et al.* 2018). In the present study, SOD level was found to increase significantly during winter from 0 day to 30th day and further to 60th day in group C. When the layers were supplemented with chromium picolinate in group CR, SOD was found to decrease significantly after 30 days of treatment (Table 2). In group CR, SOD level however increased after 60 days of treatment when the AT became lower in P4 than in P2 and P3 (Supplementary Table 1). In group VC, there was significant drop in SOD from 0 day to 30th day and further to 60th day. In group CRVC, SOD level was found to increase significantly on 30th day from that on 0 day. The level however decreased significantly on 60th day as compared to 30th day. The level of SOD estimated on 60th day in group CRVC was however not significantly different from that estimated on 0 day. Feeding of Brahma Rasayana during cold stress was found to decrease SOD in chicken owing to contents of antioxidants (Ramnath and Rekha 2011). The significant drop in SOD of layers during

Table 3. Egg parameters of White Leghorn chicken with or without supplementation of chromium and vitamin C

Day	Group C	Group CR	Group VC	Group CRVC	P-value
			Egg weight (g)		
0	60.03±1.96	59.73±2.64	60.58±2.37	59.15±1.41	0.97
15th	59.28±1.30	63.57±1.17	62.31±1.38	62.03±1.18	0.13
30th	55.05±1.83	60.08±2.20	58.43±2.00	57.33±0.81	0.27
45th	62.38±1.28	58.52±1.79	61.45±2.25	58.78±2.16	0.40
60th	57.40±1.98	61.57±2.69	60.97±2.01	59.70±1.81	0.54
75th	56.13±2.19	60.35±1.57	61.18±2.35	61.13±1.47	0.23
P-value	0.10	0.38	0.80	0.23	
			Egg length (mm)		
0	61.02^{c}±1.02	57.39±1.30	58.58±2.27	59.13±0.43	0.15
15th	58.89^{abc}±0.75	58.83±0.73	58.05±0.61	56.74±0.65	0.11
30th	57.70^{ab}±1.10	58.02±1.31	57.83±0.79	56.52±1.54	0.82
45th	59.32^{bc}±0.61	56.40±0.80	59.09±0.80	59.17±1.24	0.09
60th	58.01^{abc}±0.94	58.63±1.22	58.35±1.44	56.43±0.84	0.54
75th	56.00^{a}±1.15	56.46±1.17	56.79±0.61	56.39±0.84	0.95
P-value	0.04	0.39	0.70	0.14	
			Egg width (mm)		
0	40.15±0.34	41.07±0.53	40.77±0.58	40.31±0.34	0.50
15th	40.34^{A}±0.30	42.22^{B}±0.25	41.78^{B}±0.43	41.99^{B}±0.41	0.002
30th	39.82^{A}±0.74	42.05^{B}±0.36	41.36^{AB}±0.81	42.17^{B}±0.42	0.05
45th	41.99±0.57	41.75±0.36	41.84±0.35	41.29±0.61	0.77
60th	40.58±0.57	41.74±0.74	41.88±0.34	41.57±0.61	0.40
75th	39.98±0.70	41.49±0.15	41.42±1.65	41.80±0.58	0.54
P-value	0.10	0.35	0.92	0.20	

Means in the same column with different superscripts differ significantly.

peak winter was due to beneficial effects of chromium and vitamin C in ameliorating cold induced oxidative stress.

The egg weight and egg width of White Leghorn showed no significant changes during the period of cold stress with or without supplements of chromium, vitamin C and their combination (Table 3). This finding indicated no effects of cold stress and neither beneficial nor detrimental effects of supplementation of chromium, vitamin C and their combination on egg weight and egg width of White Leghorn chicken. The egg length was however found to decrease during the cold stress in group C while there was no significant change in groups CR, VC and CRVC (Table 3). This finding could be due to beneficial effects of chromium and vitamin C in alleviating cold stress induced effects on egg characteristics (Sahin and Sahin 2001).

SUMMARY

White Leghorn layer chicken experienced cold stress during December, 2019 to January, 2020 with lowest minimum AT and lowest average temperature during later part of January, 2020. Cold stress caused increase in HSP70 and SOD of White Leghorn layer chicken. Supplementation of chromium picolinate (@ 400 μg/kg feed) and vitamin C (@ 250 mg/kg of feed) alleviated cold stress and cold induced oxidative stress in White Leghorn chicken. Alleviation of stress by supplementation of chromium picolinate and vitamin C could be estimated by the measure of drop in HSP70 and SOD. Supplementation of chromium picolinate and vitamin C also had beneficial impacts on egg length during cold stress. Combination of chromium picolinate and vitamin C was best to alleviate cold stress while vitamin C was best to lower oxidative stress in layer.

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