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Supplementation of chromium and vitamin C to alleviate cold stress in white leghorn chicken

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Abstract
Sixty numbers of White Leghorn chicken layers were reared for 2 months from December, 2019 to January, 2020. Normal basal diet, chromium picolinate @400µg/kg of feed plus normal basal diet, vitamin C @250mg/kg of feed plus basal diet and chromium picolinate @400µg/kg of feed and vitamin C @250mg/kg of feed plus basal diet were provided to control, T1, T2 and T3 groups respectively. Ambient temperature and relative humidity were recorded at 6h intervals. Blood glucose, total plasma protein, albumin and total cholesterol were estimated in blood samples on 0, 30th and 60th day of treatment. The layers experienced a mild degree of cold stress with the lowest minimum ambient temperature of 8.72 ^{o}C. Blood glucose and total cholesterol increased due to cold in control but not in supplemented groups. The study indicated that chromium picolinate and vitamin C supplementation prevented cold stress induced rise in blood glucose and total cholesterol in layer chicken.

Keywords: Antioxidant, blood biochemical profile, winter

1. Introduction

Thermal stress leads to changes in the blood biochemical profile of layer chicken with detectable changes in blood glucose [1], total protein [2], albumin [3] and total cholesterol [4]. The changes in blood biochemical profile have been recorded both in heat stress [5-7] and cold stress [8, 9]. In presence of heat or cold stress corticosterone concentration increases which lead to decrease in insulin sensitivity [10]. Stress also leads to mineral and vitamin mobilization from tissues and thereby their excretion leading to vitamin and mineral deficiency (Siegel, 1995). Cr is essential for insulin action in protein, fat, and carbohydrate metabolism [11] and has been termed as "glucose tolerance factor" [12].

Chicken requires vitamin C for amino acid synthesis and mineral metabolism [13]. Heat stress was found to impair absorption and increase the dietary requirement vitamin C [14]. In presence of low or high environmental temperatures, humidity, and high productive rate ascorbic acid synthesis was inadequate [13].

There are reports on alleviation of heat stress impacts by supplementation of chromium [15-16], vitamin C [17] and their combination [18] in terms of alterations in blood biochemical parameters. There is however no data available to explain the role of chromium, vitamin C and their combination on alleviation of cold stress in White Leghorn chicken. The present study reveals beneficial effects of chromium picolinate, vitamin C and their combination in the alleviation of cold stress by the measure of certain blood biochemical profile in White Leghorn chicken.

2. Materials and Methods

The study was carried out at College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram for a period of 2 months from December, 2019 to January, 2020. 60 healthy White Leghorn layer chickens reared in Poultry Unit, Instructional Livestock Farm Complex were used in the present study. The birds were given their feeds @120 g/bird/day with an ad libitum supply of drinking water. Feeding was done two times a day. The % composition of feed provided to the birds is presented in Table 1.

2.1 Experiment

All the birds in different groups were kept in an enclosed area for duration of one week before treatment to familiarize the grouping and enclosure. The birds were divided into 4 groups *viz.*

control, T1, T2 and T3 and each group was comprised of 15 birds of either sex maintaining male to female sex ratio at 1:7. Control group was provided basal diet as described in Table 1. The supplemented groups T1, T2 and T3 were provided basal diet plus chromium picolinate @400 µg/kg of feed, basal diet plus vitamin C @250 mg/kg of feed and basal diet + chromium picolinate @400 µg/kg of feed +vitamin C @250 mg/kg of feed respectively for 2 months from 1st December, 2019 to 31st January, 2020.

Table 1: The composition of feed in the basal diet

Ingredients	Concentration (%)
Maize	64.8
Soybean meal	26.2
Fish meal	3.5
Rice bran oil	2.21
Dicalcium phosphate	1.25
Sodium chloride	0.3
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

2.2 Record of ambient temperature and relative humidity
Ambient temperature (AT) and relative humidity (RH) were recorded using WatchDog Automatic Weather Station at College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, Mizoram. AT and RH at 0, 6, 12 and 18h of the day were recorded during the whole period of study.

2.3 Blood sampling and analysis
Blood sampling was done on 0, 30 and 60th day of the experiment. 2 ml of blood samples were collected in heparin coated vials by puncturing the jugular vein of each bird. Plasma was separated by centrifugation of blood samples at 2000 rpm for 30 minutes. Blood glucose was estimated in fresh blood without any anticoagulant by using accu-chek active blood glucose monitoring system (Roche Diagnostics, GmbH, Mannheim, Germany). Plasma samples were used for estimation of total plasma protein and albumin by using diagnostic kits (Erba Diagnostics Mannheim GmbH, Germany) and total cholesterol by using diagnostic kit (Coral Clinical Systems, India).

2.4 Statistical analysis
The data were analyzed by using SPSS 16 version. One way analysis of variance was carried to find out the difference between different groups on different days of blood collection and between different days of blood collection of different groups [19].

3. Results and Discussion
The data obtained from the present study are presented and discussed by collating with the available literature.

3.1 Air temperature and relative humidity
The lowest minimum AT of 8.72 ºC was recorded in December, 2019 and January, 2020. The average AT was found to be 14.86 ºC in December, 2019 and 16.10 ºC in January, 2020 (Table 1). The optimal temperature for poultry had been indicated to be 15 to 20 °C [20]. The higher average RH was recorded in December, 2019 with the highest maximum RH (Table 2). The optimal relative humidity for poultry had been indicated to be 60-70% [20]. The present finding of low AT and high RH during the study indicated that laying hens had experienced cold stress with high RH.

Table 2: Ambient temperature and Relative humidity during December, 2019 to January, 2020

		December, 2019	January, 2020
AT (°C)	Average	14.86	16.10
	Minimum	8.72	8.72
	Maximum	25.27	27.77
RH (%)	Average	67.46	55.90
	Minimum	22.00	19.00
	Maximum	100.0	97.10

3.2 Blood glucose
The average blood glucose (mg/dl) in different experimental groups declined from 0 day to 30th day and increased from 30th to 60th day. In the control group, the blood glucose recorded on 60th day was significantly higher than that recorded on 0 day. An increase in blood glucose could be due to cold stress as previous studies indicated an increase in blood glucose during cold stress [8, 9]. However, in T1, T2 and T3 there was no significant rise in blood glucose on 60th day as compared to that recorded on 0 day. The difference in observation of blood glucose on 60th day in T1, T2 and T3 could be because of supplementation of chromium picolinate, vitamin C and their combination. A similar observation was also reported by [15]. Chromium is a component of chromodulin that plays roles in insulin affecting carbohydrate metabolism via insulin [20]. The supplemented chromium and vitamin C must have reduced blood glucose via activation of insulin and thereby increasing insulin uptake in tissues.

Table 3: Blood glucose (mg/dl) of White Leghorn layers supplemented with chromium picolinate and vitamin C

Day	Control	T_1	T_2	T_3	P value
0	192.83 ± 3.79^{b}	199.00 ± 5.85^{b}	199.17 ± 8.57^{b}	190.50 ± 3.53^{b}	0.64
30	159.67 ± 7.25^{a}	140.33 ± 6.03^{a}	139.67 ± 5.58^{a}	133.50 ± 24.95^{a}	0.57
60	$211.00 \pm 3.81^{c}_{B}$	$199.17 \pm 8.08^{b}_{AB}$	$187.17 \pm 3.38^{b}_{A}$	$204.67 \pm 3.25^{b}_{B}$	0.02
P value	0.000	0.000	0.000	0.009	

^{a,b,c} Means in the same column with different superscripts differ significantly.
$_{A, B}$ Means in the same row with different subscripts differ significantly.

3.3 Total plasma protein
The present study revealed no significant difference in level of total protein in different groups. It indicated that total protein of chicken did not change during cold. There was no change in total protein with supplementation of chromium picolinate, vitamin C and their combination during winter. Total protein was however found to increase on supplementation of chromium during heat stress in broilers [4, 7, 21].

Table 4: Total plasma protein (g/dl) of White Leghorn layers supplemented with chromium picolinate and vitamin C

Day	Control	T_1	T_2	T_3
0	5.68±0.55	5.38±0.51	6.07±0.58	5.78±0.91
30	6.98±0.61	5.96±0.53	5.87±0.36	6.41±0.40
60	6.40±0.77	6.11±0.61	6.26±0.43	5.75±0.65
P-value	0.358	0.643	0.820	0.669

3.4 Albumin
The average albumin (g/dl) in the current study showed no significant difference in albumin of control and T2 group. The albumin of T1 showed a significant increase from 0 day to 30th day ($P<0.05$) and remained stable till 60th day. It indicated that albumin increased with supplementation of chromium picolinate. During heat stress however, chromium supplementation was found to lower albumin in chicken [5]. In T3, albumin level increased on 30th day. The increase in albumin could be due to the supplementation of chromium and vitamin C together. However, prolong feeding had no significant effect on albumin in the present study. Rouhalamini and Salarmoin [3] also reported a significant increase of albumin in chromium supplemented Japanese quail during heat stress.

Table 5: Albumin (g/dl) of White Leghorn layers supplemented with chromium picolinate and vitamin C

Day	Control	T_1	T_2	T_3
0	2.27±0.20	1.89±0.09^a	2.06±0.10	1.89±0.19^a
30	2.94±0.29	2.50±0.15^b	2.34±0.18	2.46±0.14^b
60	2.38±0.28	2.10±0.17^b	1.98±0.13	1.91±0.16^a
P-value	0.168	0.015	0.175	0.027

^{a,b,c} Means in the same column with different superscripts differ significantly.

3.5 Total cholesterol
The average total cholesterol (mg/dl) in different experimental groups declined from 0 day to 30th day and increased from 30th to 60th day. In control group, the increase in total cholesterol on 60th day was significantly higher than that recorded on 0 day. Increase in total cholesterol could be due to cold stress as previous studies indicated increase in total cholesterol during cold stress [8, 9]. However, in T1, T2 and T3 there was no significant increase in total cholesterol on 60th day as compared to that recorded on 0 day. The difference in observation of total cholesterol on 60th day in T1, T2 and T3 could be because of supplementation of chromium picolinate, vitamin C and their combination during winter. Lowering of total cholesterol by chromium and vitamin C during heat stress had also been reported [2, 5, 22]. Chromium has been shown to involve in glucose utilization for lipogenesis and glycogen formation [20, 22]. Several studies indicated that combination of chromium and vitamin C led to more significant changes in lipid profile [18].

Table 6: Total cholesterol (mg/dl) of White Leghorn layers supplemented with chromium picolinate and vitamin C

Day	Control	T_1	T_2	T_3
0	102.20±3.60^a	153.75±6.55^b	149.32±7.80	152.48±16.77^b
30	133.89±12.48^b	79.57±4.91^a	120.39±8.63	95.95±11.64^a
60	149.98±6.75^b	171.41±15.77^b	148.71±10.70	127.19±9.46^{ab}
P-value	0.001**	0.000**	0.059^{NS}	0.015*

^{a,b} Means in the same column with different superscripts differ significantly.

4. Conclusion
The present study indicated some degree of cold stress experienced by White Leghorn chicken from December, 2019 to January, 2020. Winter stress caused increase in blood glucose and total cholesterol. Supplementation of chromium picolinate, vitamin C and their combination prevented rise in blood glucose and total cholesterol. Chromium picolinate, vitamin C and their combination could alleviate cold stress effects in White Leghorn chicken.

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