International Journal of Chemical Studies 2018; 6(4): 3381-3386

International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 3381-3386

Received: 23-05-2018
Accepted: 30-06-2018

Pragati Patel
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Aditya Mishra
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

RPS Baghel
Dean, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur

Amir Amin Sheikh
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Correspondence
Pragati Patel
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Effect of different levels of organic and inorganic chromium supplementation on oxidative stress, blood parameters and growth performance in broilers

Pragati Patel, Aditya Mishra, RPS Baghel and Amir Amin Sheikh

Abstract
Poultry production is one of the rapidly growing sub sectors among the livestock sector in India. Advances in broiler chicken genetics, nutrition and management have resulted in improved performance of birds. Hence, in the present investigation we explored the use organic chromium to modulate oxidative stress and enhance production performance of broiler chickens. Total 96 day old Cobb broiler chicks were used in the experiment. Broilers were divided into 4 groups and each group consisting of 24 birds in 3 replicates. T1 group was kept as control. T2, T3, T4 group was supplemented with chromium (2 mg/kg of feed) from inorganic, 50 % inorganic + 50 % organic and organic form respectively. Mean plasma superoxide dismutase, glutathione peroxidise and catalase concentration were non significantly different in all groups. Significantly ($p<0.05$) higher mean plasma TBA value was found in control group. No significant difference was found for mean plasma glucose level between all groups. Mean total protein, albumin and globulin were significantly ($p<0.05$) higher in T4. Mean body weight gain, feed intake, FER and PI were significantly ($p<0.05$) higher in T4. In the present investigation organic Cr supplementation showed a better antioxidant status, which could be translated into better production performance and lower mortality.

Keywords: Oxidative stress, cobb broiler, organic chromium

Introduction
Poultry production is one of the most rapidly growing sub sector among the livestock sectors in India. Advances in broiler chicken genetics, nutrition and management have resulted in improved performance of birds. Trace minerals are essential to sustain production besides regulating anti-oxidant and immune systems in chicken. Zinc (Zn), selenium (Se) and chromium (Cr) act as catalysts in many enzyme and hormone systems [1]. Conventionally, inorganic minerals are used in chicken diet, because they are cost- effective and readily available, but are relatively inferior to organic minerals due to poor bioavailability [2]. Improved performance, anti-oxidant and immune responses were reported in chicken fed higher concentrations of these trace minerals. To mature these benefits, trace minerals are supplemented at higher concentrations in diet. However, such higher levels of inorganic trace mineral (ITM) were reported to be detrimental to performance of birds due to reduced absorption and also increase excretion of these minerals into the environment. Higher concentrations of inorganic TM (ITM) will interfere with each other which may cause either deficiency or toxicity. However, organic form of TM (OTM) will not interfere with other minerals due to different pathway of absorption through intestinal wall [1].
Chromium (Cr) has been considered as an anti-stress factor to ameliorate the effects of environmental stress. Chromium (Cr) is an essential element required for carbohydrate, fat and protein metabolism. The beneficial effect of chromium in animal health is well documented for its role as an integral component of the glucose tolerance factor (GTF). Chromium supplementation in diet has been related to increased protein deposition, with decrease in muscle fat. Dietary chromium supplementation increases the growth rate, feed efficiency and improves meat yield as well as carcass quality with reduced carcass fat in broilers [3]. Hence, the present study has been designed to study the influence of organic source of chromium supplementation on oxidative stress, blood parameters and growth performance in broilers.

Material and Methods

The research was carried out in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.).

Experimental Birds

Ninety six (96) day old Cobb broiler chicks were procured from Private hatcheries of Jabalpur. The birds were maintained in the battery cage system in a well ventilated room in the poultry experimental unit at college with prior permission from Institutional Animal Ethics Committee. Broilers were divided into 4 groups and each group consisting of 24 birds in 3 replicates. T1 group was kept as control. T2, T3, T4 group was supplemented with chromium (2 mg/kg of feed) from inorganic, 50 % inorganic + 50 % organic and organic form respectively.

Diets were formulated as per [4] specifications. Feed-grade sulphate salts of Mn, Zn, Fe and Cu were used, in the control diet (CD) while in group T2 inorganic Cr was supplemented in the form of dichromate (potassium dichromate). The organic forms of Cr (Biochrome) were generous gift from Alltech Biotechnology Pvt. Ltd., Bengaluru, India. A concentration of Cr in the organic Biochrome was 0.1 %. A CD with the ITM was fed as control group.

Experimental Design

Groups	Replicates	No. of Broilers	Treatments
T_1 (n=24)	$T_1 R_1$	8	Basal diet
	$T_1 R_2$	8	
	$T_1 R_3$	8	
T_2 (n=24)	$T_2 R_1$	8	Basal diet + Inorganic chromium @ 2 mg/kg of feed
	$T_2 R_2$	8	
	$T_2 R_3$	8	
T_3 (n=24)	$T_3 R_1$	8	Basal diet + 50% Inorganic chromium @ 1 mg/kg of feed + 50% Organic chromium @ 1 mg/kg of feed
	$T_3 R_2$	8	
	$T_3 R_3$	8	
T_4 (n=24)	$T_4 R_1$	8	Basal diet + Organic chromium @ 2 mg/kg of feed
	$T_4 R_2$	8	
	$T_4 R_3$	8	

Table 1: Ingredients and composition of broiler ration

Ingredients	Starter %	Finisher %
Maize	43.36	57.30
Soybean meal	43.90	33.10
Soybean oil	8.74	5.61
Common Salt	0.40	0.40
DL- Methionine	0.185	0.175
Di-Calcium Phosphate	1.80	1.80
Limestone Powder	1.37	1.37
Supplements (Vitamins supplement and feed additives)	0.245	0.245

*Trace mineral Premix: Mn-55,I-0.4, Fe-56 and Cu-4kg-1
**Vitamin premix: Vitamin A-8250 IU, Vitamin D_3- 1200 IU, Vitamin k-1mg, Vitamin E-40 IU, Vitamin B1-2mg, Vitamin B_2-4mg, Vitamin B_{12}-10mg, Percent of values specified by NRC, 1994,
*** Calculated

Collection of Samples

Blood samples (approx. 2 ml) were collected on day 21, 28 and 35 from each bird of all experimental groups. Blood were collected from the wing vein. The blood samples collected in heparinized polypropylene tubes (20 IU heparin/ml of blood) were kept in the ice bucket and carried back to the laboratory immediately. In the laboratory, all the blood samples were centrifuged at 3000 rpm for 30 min and plasma was separated. Plasma obtained was kept in the labeled storage vials of 2 ml capacity and stored at -20°C till analysis of oxidative stress and biochemical parameters.

The quantitative estimation of chicken superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) levels were analyzed by the sandwich Elisa technique. Lipid peroxidation was determined by a micro method for TBA reactive substances (TBARS) given by [5]. The plasma glucose concentration was estimated by Trinder's method [6] using diagnostic kits. The concentration was expressed in mg/dl of blood glucose. Total protein was estimated by Biuret method [7] using the diagnostic kits. The concentration of total protein was expressed as g/dl of serum. Plasma albumin concentration was estimated as per method described by [8] using diagnostic kits procured from Erba Diagnostics, Mannheim Gmbh, Germany. The concentration was expressed in g/dl of albumin. Plasma globulin concentration was also estimated by using diagnostic kits. The concentration was expressed in g/dl of globulin. All diagnostic kits were procured from Erba Diagnostics, Mannheim Gmbh, Germany. Automatic biochemistry analyzer was used for the determination of all biochemical parameters.

Body weight was recorded individually at weekly interval till 5 week of age by weighing all the birds in each treatment group using electronic weighing balance in the morning hours before feeding. Feed intake was recorded by weighing the offered feed and residual feed on weekly basis. Weekly feed consumption of broilers replicate was recorded till the end of week. The feed consumed was determined by subtracting the residual feed from total feed offered during each of the period. Feed efficiency ratio was calculated on the basis of body weight gain and feed intake in weekly interval basis of the experiment. Performance index was calculated as per the formula proposed by [9]. Mortality was recorded on daily basis and the mortality rate was calculated for the period from 0 to 5 weeks. The recorded data was statistically analyzed using Completely Randomized Design. Various conditions and treatment groups were compared by using Duncan Multiple Range test (DMRT).

Results and Discussion

Oxidative Stress

Oxygen radicals and other reactive oxygen species (ROS) are widely produced during cell metabolism. ROS can react with double bonds of polyunsaturated fatty acids (PUFAs) to yield lipid hydroperoxides and cause tissue damage. However, there are some defense mechanisms to remove ROS in the cells. Trace minerals are involved directly or indirectly in the formation of many enzymes against ROS.

Superoxide dismutase, glutathione peroxidise and catalase

The mean plasma superoxide dismutase, glutathione peroxidise and catalase concentration in broilers has been presented in Table 2, 3 and 4 respectively. The statistical analysis showed non-significant difference between all the groups. But numerically higher concentration of these enzymes was found in T4 group which were supplemented with organic chromium.

Present findings are in accordance with earlier work of [1]. They reported that improvement in activity of SOD with supplementation of Se, Zn or Cr as compared to those fed the control diet without OTM. The activity of SOD increased with supplementation of OTM and further improvement was not observed with increase in concentration of the trace minerals in diet.

Table 2: Mean plasma superoxide dismutase (SOD) concentration (pg/ml) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	2.803 ± 1.14	3.521 ± 0.28	3.937 ± 0.67
T2	4.194 ± 0.31	4.057 ± 0.38	4.253 ± 0.15
T3	4.512 ± 1.42	4.239 ± 0.89	4.400 ± 0.99
T4	4.664 ± 0.93	4.465 ± 0.28	4.429 ± 0.70

Table 3: Mean plasma glutathione peroxidase (ng/ ml) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	11.582 ± 1.64	10.586 ± 0.94	10.521 ± 1.19
T2	12.323 ± 2.33	11.437 ± 0.86	11.836 ± 1.05
T3	12.514 ± 2.10	12.465 ± 2.65	11.867 ± 0.96
T4	12.795 ± 0.49	13.457 ± 2.99	14.274 ± 1.43

Table 4: Mean plasma catalase (CAT) concentration (ng/ ml) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	6.360 ± 4.43	8.566 ± 0.68	9.350 ± 1.18
T2	11.719 ± 3.52	9.129 ± 2.37	11.285 ± 1.09
T3	12.257 ± 2.31	10.659 ± 0.57	12.373 ± 1.17
T4	12.377 ± 1.59	11.390 ± 1.15	12.619 ± 1.15

Thio-barbituric acid value

The mean plasma glucose concentration in broilers has been presented in Table 5. The mean plasma thio-barbituric acid value showed significant difference ($p<0.01$) between all the groups. The maximum plasma thio-barbituric acid value was observed in T1 group whereas, minimum plasma thio-barbituric acid value was observed in T4 group during experimental period. The decrease in plasma MDA concentration could be due to the increased activity of SOD, Gpx and CAT and decrease in the damage of tissues by OTM [1].

[10] Studied the measurement of malondialdehyde as an oxidative stress indicator in the serum of the tested bird groups and revealed that significant increase ($p<0.001$) in its concentration in the heat stressed control group as compared to that of the thermoneutral control one. On the other hand, supplemental diets ameliorated these deleterious effects as clearly seen by a significant decrease ($p<0.001$) in malodialdehyde concentration in vitamins "E+C", "Zn+Se" and Cr groups. [11] Reported that dietary supplementation of chromium, selenium and vitamin C separately or in combination decreased MAD concentration ($p<0.05$) in heated-stressed birds as compared with the control group. [1] Found that the lipid peroxidation was reduced ($p<0.05$) with supplementation of Se or Cr compared to the broilers fed the control diet, while the lipid peroxidation in Zn supplemented groups was intermediate.

Contrary to these findings, [12] Studied that organic trace mineral supplementation enhances local and systemic innate immune responses and modulates oxidative stress in broiler chickens. They reported that lipid peroxidation analysis showed no difference in plasma MDA level between control and OTM treatment ($p>0.05$) groups.

Table 5: Plasma thiobarbituric acid value (µmol/ml) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	$3.49^a \pm 0.01$	$3.36^a \pm 0.05$	$3.21^a \pm 0.01$
T2	$2.68^b \pm 0.02$	$2.59^b \pm 0.03$	$2.51^b \pm 0.02$
T3	$2.69^b \pm 0.02$	$2.58^b \pm 0.04$	$2.42^b \pm 0.04$
T4	$2.66^b \pm 0.02$	$2.47^b \pm 0.02$	$2.37^c \pm 0.01$

Biochemical Parameters

Plasma Glucose Concentration

The mean plasma glucose concentration in broilers has been presented in Table 6. The statistical analysis showed non significant ($p>0.05$) difference in plasma glucose concentration among the different treatment groups but the pattern of results indicates minimum concentration of plasma glucose was observed in organic chromium supplemented group of broilers. The lower circulatory glucose concentration in the Cr supplemented birds was perhaps indicative of an increased turnover rate and utilization of glucose at the tissue level. [13] Reported Cr as a cofactor for insulin activity and that it is necessary for normal glucose utilization and healthy animal growth. Insulin regulates metabolism of carbohydrate, fat and protein, stimulating amino acid uptake and protein synthesis as well as glucose utilization in tissues [14]. Present findings are in agreement to previous findings.

[15] Observed significant decrease in serum glucose in Japanese quails fed with chromium picolinate. [16] Reported significant decreased serum glucose in laying quails fed with chromium picolinate. [17] Also observed significant decrease in serum glucose by feeding organic chromium at different doses which is in agreement to present finding. [11] Reported that dietary supplementation of chromium, selenium and vitamin c separately or in combination, decreased glucose ($p<0.05$) in heated-stressed birds as compared with the control group.

Table 6: Mean plasma glucose (mg/dl) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	258.00 ± 12.49	246.33 ± 09.52	250.33 ± 18.49
T2	239.33 ± 04.84	223.33 ± 24.91	224.00 ± 34.50
T3	227.67 ± 08.17	215.67 ± 12.91	223.00 ± 16.50
T4	227.33 ± 22.74	212.00 ± 16.64	3 ± 11.46

Total protein, albumin and globulin concentration

The mean total plasma protein, albumin and globulin concentration of broilers has been presented in Table 7, 8 and 9 respectively. The mean total plasma protein, albumin and globulin concentration showed significant difference ($p<0.01$) betwaeen all the groups.

The probable reason behind increased serum concentration of total protein, albumin and globulin may be due to the involvement of Cr in protein metabolism. Chromium is thought to have a role in nucleic acid metabolism because it increased in the stimulation of amino acid incorporation into liver protein. It is expected that Cr might have effects on circulating proteins because of its role in protein synthesis. The increase in serum albumin may be due to increased amino acid synthesis in the liver, suggesting that Cr may improve amino acid synthesis. [13] Reported Cr as a cofactor for insulin activity and that it is necessary for normal glucose utilization and healthy animal growth. Insulin regulates metabolism of carbohydrate, fat and protein, stimulating amino acid uptake and protein synthesis as well as glucose utilization in tissues [14].

The present findings were in agreement with [16] who reported that positive changes were observed in serum total protein by feeding chromium picolinate (organic Cr) to Japanese quails. [18] Observed non significant difference in the A:G ratios at 28 d ($p>0.05$), but the A:G ratio was significantly higher at 42 d for the 1,200 ppb of organic Cr group as compared to the control and other groups ($p< 0.01$). The increase in the A:G ratio in the 1,200 ppb of organic Cr group observed at 42 d. [19] Reported that the higher value in serum total protein was observed by feeding of chromium picolinate to broiler chicks. [10] Reported that supplementation of Zn + Se and Cr significantly increased total protein levels in plasma. [11] Also reported that separately or in combination, supplemental Cr, Se or Vitamin C increased serum concentrations of total protein, albumin and globulin which are in agreement with present findings.

Contrary to these findings [12] Reported that the overall analysis of total protein, fibrinogen, total protein/fibrinogen ratio, albumin, globulin did not show any difference among OTM treatments ($p >0.05$) and control group.

Table 7: Mean plasma total protein (g/dl) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	$2.53^{d} \pm 0.02$	$2.53^{d} \pm 0.04$	$2.45^{d} \pm 0.08$
T2	$3.65^{c} \pm 0.09$	$3.64^{c} \pm 0.10$	$3.58^{c} \pm 0.13$
T3	$3.96^{b} \pm 0.06$	$3.96^{b} \pm 0.11$	$3.94^{b} \pm 0.08$
T10	$4.83^{a} \pm 0.19$	$4.83^{a} \pm 0.09$	$4.83^{a} \pm 0.19$

Means bearing different superscripts within same row differ significantly ($p<0.01$).

Table 8: Mean plasma albumin (g/dl) in broilers at different intervals

Period / Treatment	21st day	28th day	35th day
T1	$1.66^{c} \pm 0.03$	$1.64^{c} \pm 0.04$	$1.64^{c} \pm 0.07$
T2	$1.98^{b} \pm 0.09$	$1.94^{b} \pm 0.08$	$1.9^{ab} \pm 0.02$
T3	$2.07^{a} \pm 0.08$	$2.07^{ab} \pm 0.04$	$2.04^{ab} \pm 0.05$
T4	$2.14^{a} \pm 0.06$	$2.16^{a} \pm 0.03$	$2.15^{a} \pm 0.06$

Means bearing different superscripts within same row differ significantly ($p<0.01$).

Table 9: Mean plasma globulin (g/dl) in broilers at different intervals

Period Treatment	21st day	28th day	35th day
T1	$0.83^{c} \pm 0.01$	$0.86^{c} \pm 0.08$	$0.71^{c} \pm 0.09$
T2	$1.59^{b} \pm 0.11$	$1.64^{b} \pm 0.05$	$1.59^{b} \pm 0.11$
T3	$1.82^{b} \pm 0.11$	$1.83^{b} \pm 0.12$	$1.85^{b} \pm 0.04$
T4	$2.64^{a} \pm 0.15$	$2.61^{a} \pm 0.11$	$2.59^{a} \pm 0.16$

Means bearing different superscripts within same row differ significantly ($p<0.01$).

Production performance parameters of Broilers

Body weight gain

Significant difference ($p<0.01$) was observed in body weight gain between all the groups. The maximum body weight gain (1900.87 ± 25.11 g) was attained in T4 group, supplemented with organic form of Cr followed by T7 group whereas, minimum body weight gain (1403.29 ± 21.24 g) was attained in T1 (control group). It may be due to the use of organic minerals which can improve intestinal absorption of trace elements as they reduce interference from agents that form insoluble complexes with the ionic trace elements and thereby enhancing their bioavailability and body weight gain.

Present findings were in agreement with the results of [1] they reported that supplementation of Cr as organic form significantly increased ($p<0.05$) body mass gain as compared to those fed the control diet.

The present findings of higher body weight gain recorded from supplementation of organic Cr was not in agreement with the results obtained by [1] who observed non- significant difference in body weight of Vanaraja chicks fed with reduced levels of organic trace minerals (Zn, Mn, Cu, Fe, I, Se and Cr). [12] Also reported that body weight was not different in OTM treatments compared to control ($p>0.05$).

Feed Intake

Significant difference ($p<0.01$) in total feed intake (Kg) was observed between all the groups during entire experimental duration. Maximum feed intake was observed for T4 (2280.99 ± 16.21 g) group. This might be due to synergistic effect of of stress alleviating nutritional agents (OTM), they in turn may had helped in improvement of feed intake in broilers.

The present findings of significant influence of feed consumption was in agreement with the results of [20, 21] they found that improved cumulative weight gain and feed intake were observed with diet supplemented with Chromium Picolinate in heat stress birds. Similar results were also reported by [22].

Feed efficiency ratio and performance index

Feed efficiency ratio and performance index has been presented in Table 10. FER and PI both are significantly ($p<0.01$) different for all the treatment groups as compare to control group. Maximum feed efficiency ratio and performance index were observed inT4 group which were supplemented with organic form of chromium.

The present observations are in agreement with the findings of [15] they reported that increase in supplemental chromium (200, 400,800 or 1200 µg/kg Cr picolinate) resulted in increase in feed efficiency of broilers reared under heat stress. [23] reported that Cr as Cr Pic increased feed efficiency in 0 to 3wk broilers. [24] Reported that an experiment to evaluate the effect of chromium (Cr) from Cr yeast on the feed efficiency. The birds were fed with a control diet or a control diet supplemented with Cr at a level of 300, 500 µg/kg Cr. The supplementation of 500 µg/kg Cr increased feed efficiency ($p<0.05$).

Table 10: Production performance of broilers

Treatment \ Parameter	Wt. gain (g)	FI (g)	FER	PI
T1	$1403.29^{d} \pm 21.24$	$2161.29^{b} \pm 52.22$	$0.65^{d} \pm 0.01$	$921.17^{d} \pm 28.09$
T2	$1480.45^{c} \pm 24.12$	$2096.54^{b} \pm 75.66$	$0.70^{c} \pm 0.01$	$1057.12^{c} \pm 33.80$
T3	$1684.45^{b} \pm 26.19$	$2170.21^{b} \pm 16.18$	$0.78^{b} \pm 0.01$	$1323.36^{b} \pm 40.56$
T4	$1900.87^{a} \pm 25.11$	$2280.99^{a} \pm 16.21$	$0.83^{a} \pm 0.01$	$1594.65^{a} \pm 41.50$

Means bearing different superscripts within same row differ significantly ($p<0.01$).

Conclusion

Supplementation of organic form of Cr (2 mg/kg) improved anti-oxidant status of the broilers (reduced LP and increased activity of SOD, GSH Px and CAT) and production performance of broilers. Organic Cr is found to be necessary for glucose utilization in tissues and it also enhances protein synthesis, thus helps in faster body growth in broilers.

References

1. Rama Rao SV, Prakash B, Raju MVLN, Panda AK, Kumari RK, Reddym EPK. Effect of supplementing organic forms of zinc, selenium and chromium on performance, anti-oxidant and immune responses in broiler chicken reared in tropical summer. Biological Trace Element Research. 2016; 172(2):511-520.
2. Virden WS, Yeatman JB, Barber SJ, Willeford KO, Ward TL, Fakler TM. *et al.* Immune system and cardiac functions of progeny chicks from dams fed diets differing in zinc and manganese level and source. Poultry Science, 2004; 83:344-351.
3. Samanta S, Haldar S, Bahadur V, Ghosh TK. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. Journal of Science Food and Agriculture. 2008; 88:787-796.
4. NRC. Nutrient Requirement of Poultry (9th Rev.Edn.). National Research Council, National Academy Press, Washington, DC, 1994, 20418.
5. Niehaus WG, Samuelson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. European Journal of Biochemistry, 1968; 6(1):126-130.
6. Pileggi VJ. Szuskeiweiz. Carbohydrates.In: Henry Richard J C, Canon Donald, and W Winkelman, James (eds.) Clinical Chemistry. Principles and Techniques. 2nd Edn. Harper and Row Publishers, New York. 1974, 1265-1325.
7. Tietz NW. Text book of Clinical Chemistry, W.B. Saunders publishing Co., Philadelphia PA, 1986, 579.
8. Doumas BT, Arends R, Pinto PC. Standard Methods of Clinical Chemistry, 7th Edn, Academic Press, Chicago, 1972, 175-189.
9. Bird HR. Performance index of growing chickens. Poultry Science, 1995; 34:1163-1164.
10. Tawfeek SS, Hassanin KMA, Youssef IMI. The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. JWPR. 2014; 4(1):10-19.
11. Attia kh M, Tawfeek FA, Mady MS, Assar MH. Effect of dietary chromium, selenium and vitamin c on productive performance and some blood parameters of local strain

dokki-4 under Egyptian summer conditions. Egyptian Poultry Science. 2015; 35(1):311-329.
12. Echeverry H, Yitbarek A, Munyaka P, Alizadeh M, Cleaver A, Camelo-Jaimes G. *et al*. Organic trace mineral supplementation enhances local and systemic innate immune responses and modulates oxidative stress in broiler chickens. Poultry Science. 2016; 95:518-527.
13. Rosebrough RW, Steele NC. Effects of supplemental dietary chromium or nicotic acid on carbohydrate metabolism during basal, starvation and refeeding periods in poultry. Poultry Science. 1981; 60:407-41.
14. Sahin K, Kucuk O, Sahin N. Effects of dietary chromium picolinate supplementation on performance, insulin and corticosterone in laying hens under low ambient temperature. Journal of Animal Physiology and Animal Nutrition. 2001; 85:142-147.
15. Sahin K, Ozbey O, Onderci M, Cikim G, Aysondu MH. Chromium supplementation can alleviate negative effects of heat stress on egg production, egg quality and some serum metabolites of laying Japanese quail. Journal of Nutrition, 2002; 132(6):1265-1268.
16. El-Samra HA, Abo-Egla, Kalaba ZM, Tolba AAH, El-Deeb MAI. Alleviating adverse effects of heat stress by using organic selenium and chromium for local laying hens. Journal of Animal Poultry Production. 2014; 5(7):397-411.
17. Bahrami A, Moeini MM, Ghazi SH, Targhibi MR. The effect of different levels of organic and inorganic chromium supplementation on immune function of broiler chicken under heat-stress conditions. Journal of Applied Poultry Research. 2012; 21:209-215.
18. Ebrahimnazhad Y, Ghanbari S. The effect of dietary chromium supplementation on blood biochemical parameters of broiler chicks. Greener Journal of Biological Science. 2014; 4(3):98-102.
19. Naghieh A, Toghyani M, Gheisari AA, Saeed SE, Mirzazade H. Effect of different sources of chromium on performance and immune responses of broiler chicks. Journal of Animal Veterinary Advances. 2010; 9:354-358.
20. Moeini MM, Bahrami A, Ghazi S, Targhibi MR. The effect of different levels of organic and inorganic chromium supplementation on production performance, carcass traits and some blood parameters of broiler chicken under heat stress condition. Biology of Trace Element and Research, 2011; 144:715-724.
21. Hamidi O, Mohammad C, Hasan G, Ali AS, Hassan MK. Effects of chromium (iii) picolinate and chromium (iii) picolinate nanoparticles supplementation on growth performance, organs weight and immune function in cyclic heat stressed broiler chickens. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2016; 22(3):373-380.
22. Lee DN, Wu FY, Cheng YH, Lin RS, Wu PC. Effects of dietary chromium picolinate supplementation on growth performance, feed efficiency and immune responses of broilers. Asian-Australian Journal of Animal Science, 2003; 16:227-233.
23. Nascimento ST, Silva IJOD, Mourao GB, Castro ACD. Bands of respiratory rate and cloacal temperature for different broiler chicken strains. R. Bras. Zootechnology. 2012; 41:1318-1324.
24. Kroliczewska BB, Zawadzki W, Dobrzanski, Z, Kaczmarekoliwa A. Changes in selected serum parameters of broiler chicken fed supplemental chromium. Journal of Animal Physiology and Animal Nutrition. 2004; 88(11-12):393-400.