Effect of Chelated and Inorganic Zinc, Selenium and Chromium on Antioxidant Status, Biochemical and Production Parameters in Broiler

Pragati Patel[1], Aditya Mishra[1], A.P. Singh[2], A.K. Singh[1]

10.18805/ijar.B-3924

ABSTRACT

In the present investigation we explore the use of organic and inorganic zinc, selenium and chromium to modulate antioxidant defense system, biochemical status and enhance production response of Broiler. Total 312 day old Cobb broiler chicks were used in the experiment. The results indicate that mean plasma superoxide dismutase, glutathione peroxidise and glucose concentration were non-significantly different in all groups. Significantly ($p<0.05$) higher mean plasma catalase concentration and TBA value was found in T13 and control group respectively. Mean plasma total protein, albumin and globulin were significantly ($p<0.01$) higher in organic Zn, Se and Cr (T13) followed by organic Cr (T10) supplemented group. Mean body weight gain, feed intake, FER and PI were significantly ($p<0.01$) higher in organic Zn, Se and Cr (T13) followed by organic Se (T7) supplemented group. In the present investigation organic Zn, Se and Cr supplementation showed systemic effects with a better biochemical status, which could be translated into better production performance and lower mortality.

Key words: Chromium, Inorganic, Organic, Selenium, Zinc.

INTRODUCTION

A single or blend of organic trace mineral (OTM) in diets has been shown to have multiple beneficial effects in broiler production. Poultry production is one of the rapidly growing sub sectors among the livestock sector in India. Besides contributing markedly to GDP and earning foreign exchange, it also produces good quality animal protein at an affordable price to meet the protein demand of human population by exploiting the genetic potential and adopting the standard scientific managemental and nutritional practices. Utilization of effective trace minerals in diet will protect the biological system (Echeverry *et al.,* 2016).

Zinc (Zn), selenium (Se) and chromium (Cr) act as catalysts in many enzyme and hormone systems. 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. 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 (Rama Rao *et al*., 2016).

Supplementation of zinc (Zn) and selenium (Se) in broiler diet is of particular interest because they function predominantly as catalyst in many enzyme and hormone system that are associated with growth. The Zn was found to be essential for normal functioning of the immune system. Chromium supplementation in diet has been related to increased protein deposition, with decrease in muscle fat. Immunological function has been enhanced by trivalent Cr and its effects seem more pronounced during times of stress.

[1]Department of Veterinary Physiology and Biochemistry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
[2]Department of Animal Biotechnology, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.

Corresponding Author: Aditya Mishra, Department of Veterinary Physiology and Biochemistry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
Email: amishra5@yahoo.co.in

How to cite this article: Patel, P., Mishra, A., Singh, A.P. and Singh, A.K. (2021). Effect of Chelated and Inorganic Zinc, Selenium and Chromium on Antioxidant Status, Biochemical and Production Parameters in Broiler. Indian Journal of Animal Research. 55(1): 52-59. DOI: 10.18805/ijar.B-3924.

Submitted: 18-09-2019 **Accepted:** 16-03-2020 **Online:** 15-05-2020

Hence, in the present investigation we explore the use of organic and inorganic zinc, selenium and chromium to modulate antioxidant defense system, biochemical status and enhance production response of Broiler.

MATERIALS AND METHODS

The research was carried out on March, 2018 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

Three hundred and twelve (312) day old Cobb broiler chicks were procured from Private hatcheries of Jabalpur. Broilers were kept in closed ventilated system for 35 days during the experimental period. 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 13 groups and each group consisting of 24 birds in 3 replicates.

Diets were formulated as per NRC (1994) specifications. Feed-grade sulphate salts of Mn, Zn, Fe and Cu were used in the control diet (CD), while in inorganic treatment group Se and Cr were used in the form of selenite (sodium selenite) and dichromate (potassium dichromate), respectively. The organic forms of Se, Zn and Cr (Sel-Plex 2000, Bioplex zinc and Biochrome, respectively) were generous gift from Alltech Biotechnology Pvt. Ltd., Bengaluru, India. Concentrations of Zn, Se and Cr in the above organic TM premixes were 15, 0.2 and 0.1%, respectively. (Table 1).

Collection of samples

- Blood samples (approx. 2 ml) were collected on day 21, 28 and 35 from each bird of all experimental groups for different enzymes and biochemical analysis.

Experimental design

Groups	Replicates	No. of Broilers	Treatments
	T_1R_1	8	
T_1 (n=24)	T_1R_2	8	Basal diet
	T_1R_3	8	
	T_2R_1	8	
T_2 (n=24)	T_2R_2	8	Basal diet + Inorganic zinc @ 40 mg/kg of feed
	T_2R_3	8	
	T_3R_1	8	
T_3 (n=24)	T_3R_2	8	Basal diet + 50% Inorganic zinc @ 20 mg/kg of feed+ 50% Organic zinc @ 20 mg/kg of feed
	T_3R_3	8	
	T_4R_1	8	
T_4 (n=24)	T_4R_2	8	Basal diet + Organic zinc @ 40 mg/kg of feed
	T_4R_3	8	
	T_5R_1	8	
T_5 (n=24)	T_5R_2	8	Basal diet + Inorganic selenium @ 0.30 mg/kg of feed
	T_5R_3	8	
	T_6R_1	8	
T_6 (n=24)	T_6R_2	8	Basal diet + 50% Inorganic selenium @ 0.15 mg/kg of feed + 50% Organic selenium @ 0.15 mg/kg of feed
	T_6R_3	8	
	T_7R_1	8	
T_7 (n=24)	T_7R_2	8	Basal diet + Organic selenium @ 0.30 mg/kg of feed
	T_7R_3	8	
	T_8R_1	8	
T_8 (n=24)	T_8R_2	8	Basal diet + Inorganic chromium @ 2 mg/kg of feed
	T_8R_3	8	
	T_9R_1	8	
T_9 (n=24)	T_9R_2	8	Basal diet + 50% Inorganic chromium @ 1 mg/kg of feed + 50% Organic chromium @ 1 mg/kg of feed
	T_9R_3	8	
	$T_{10}R_1$	8	
T_{10} (n=24)	$T_{10}R_2$	8	Basal diet + Organic chromium @ 2 mg/kg of feed
	$T_{10}R_3$	8	
	$T_{11}R_1$	8	
T_{11} (n=24)	$T_{11}R_2$	8	Basal diet + Inorganic zinc @ 40 mg/kg of feed + Inorganic selenium @ 0.30 mg/kg of feed + Inorganic chromium @ 2 mg/kg of feed
	$T_{11}R_3$	8	
	$T_{12}R_1$	8	
T_{12} (n=24)	$T_{12}R_2$	8	Basal diet + 50% Inorganic zinc @ 20 mg/kg of feed+ 50% Organic zinc @ 20 mg/kg of feed + 50% Inorganic selenium @ 0.15 mg/kg of feed + 50% selenium @ 0.15 mg/kg of feed + 50% Inorganic chromium @ 0.15 mg/kg of feed + 50% chromium @ 0.15 mg/kg of feed
	$T_{12}R_3$	8	
	$T_{13}R_1$	8	
T_{13} (n=24)	$T_{13}R_2$	8	Basal diet + organic zinc @ 40 mg/kg of feed + organic selenium @ 0.30 mg/kg of feed + organic chromium @ 2 mg/kg of feed
	$T_{13}R_3$	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.

1. Oxidative stress parameters

1.1 Superoxide dismutase, Glutathione peroxidase and Catalase

The quantitative estimation of chicken superoxide dismutase, chicken glutathione peroxidase and catalase were analyzed by the sandwich Elisa technique.

1.2 Thio-barbituric acid value (Malondialdehyde method)

0.1 ml of plasma was treated with 2 ml of TBA 0.37%, 0.25 N HCl and 15% TCA (1:1:1) and heated in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank (Niehaus and Samuelson, 1968).

2. Biochemical parameters

All biochemical parameters including plasma glucose, total protein, plasma albumin and plasma globulin was estimated by automatic biochemistry analyzer using diagnostic kits procured from Erba Diagnostics, Mannheim Gmbh, Germany.

3. Production performance parameters

Body weight

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

Feed intake was recorded by weighing the offered feed and residual feed on weekly basis.

Feed efficiency ratio

Feed efficiency ratio was calculated on the basis of body weight gain and feed intake in weekly of the experiment. FER was calculated using following formula.

FER = Body weight gain (g) / Feed consumption (g)

Performance index

Performance index was calculated as per the formula proposed by Bird (1955).

PI = Body weight gain (g) X FER

Mortality

Mortality was recorded on daily basis and the mortality rate was calculated for the period from 0 to 5 weeks.

Statistical analysis

The recorded data was statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1994). Various conditions and treatment groups were compared by using Duncan Multiple Range test (DMRT).

RESULTS AND DISCUSSION

Oxidative stress parameters

Superoxide dismutase

The mean concentration of plasma superoxide dismutase showed non-significant difference between all the groups (Table 2). The pattern of results indicates that, the maximum concentration of plasma superoxide dismutase was observed in combination of organic mineral supplemented group (T13) whereas, in groups supplemented with separately Zn, Se or Cr, the maximum concentration of plasma superoxide dismutase was observed in organic Zn supplemented group (T4) followed by T3 than T2 group.

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	5.110 ± 0.51	4.379 ± 0.63	5.105 ± 1.19
T3	5.575 ± 0.56	4.644 ± 0.08	5.152 ±1.67
T4	5.756 ± 0.12	4.032 ± 0.38	5.377 ± 1.56
T5	4.463 ± 0.85	4.106 ± 0.13	4.385 ± 1.20
T6	4.670 ± 0.55	4.274 ± 0.39	4.444 ± 0.26
T7	4.984 ± 1.20	4.647 ±0.39	4.646 ± 0.85
T8	4.194 ± 0.31	4.057 ± 0.38	4.253 ± 0.15
T9	4.512 ± 1.42	4.239 ± 0.89	4.400 ± 0.99
T10	4.664 ± 0.93	4.465 ± 0.28	4.429 ± 0.70
T11	5.565 ± 0.84	4.789 ± 1.05	5.291 ± 1.14
T12	5.965 ± 079	5,565 ± 0.99	5.982 ± 0.17
T13	6.845 ± 0.70	6.224 ± 1.72	6.670 ± 2.15

In present investigation the level of superoxide dismutase (SOD) was increased by Zn supplementation. The probable reason for the increase might be the fact that zinc act as a structural component of SOD. Superoxide dismutase is localized in cytoplasm, which includes Cu and Zn at its catalytic site. The enzyme SOD acts as the first line of antioxidant defence by catalyzing the conversion of the superoxide anion into hydrogen peroxide and oxygen.

Present findings are in accordance with earlier work of Rama Rao *et al.* (2016). 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. In another study Suri, (2015) reported that the activity of superoxide dismutase differed significantly with variation in source of Zn supplementation and the activity of SOD ($p<0.05$) was highest at 70 ppm (5.27 units/mg protein.).

Glutathione peroxidase

The mean concentration of plasma glutathione peroxidase showed non-significant difference between all the groups (Table 3). The pattern of results indicates that the maximum concentration of plasma glutathione peroxidase was observed in organic mineral supplemented group (T13) whereas, in groups supplemented separately with Zn, Se or Cr, the maximum concentration of plasma glutathione peroxidase was observed in organic Se supplemented group (T7) followed by T6 than T5 group. The probable reason for this might be the activation of glutathione peroxidase requires small amounts of Se (selenocysteine), probably substituting sulphur in the glutathione molecule and causing development of modified enzyme GPx4. Lipid peroxidation in plasma was decreased, while activities of GPx and glutathione reductase in plasma increased linearly with Se concentration in a broiler chicken diet as reported by Rama Rao *et al*. (2013).

Catalase

On day 35 the mean concentration of plasma catalase showed significant ($p<0.01$) difference between all the groups (Table 4). Significantly ($p<0.01$) higher level of plasma catalase was found in T4 (16.265 ± 0.80 ng/ ml), T12 (17.287 ± 2.76 ng/ ml) and T13 (20.230 ± 1.66 ng/ ml) groups.

Present findings are in accordance with Rama Rao *et al.* (2013) who found that red blood cell catalase (RBCC) increased linearly ($p<0.01$) with increase in Se concentration in diet. Catalase is a heme-containing antioxidant enzyme that acts sequentially with SOD in the conversion of hydrogen peroxide to water thereby protecting the cells against hydrogen peroxide toxicity and LPO (Suri, 2015).

Thio-barbituric acid value

The mean plasma thio-barbituric acid value showed significant difference ($p<0.01$) between all the groups (Table 5). The maximum plasma thio-barbituric acid value was observed in T1 group whereas, minimum plasma thio-barbituric acid value was observed in T13 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 (Rama Rao *et al*., 2016).

Present findings were much similar to findings of Sahin *et al*. (2005). They observed linear decrease ($P<0.05$) in malondialdehyde (MDA) concentration in heat stressed Japanese quails by addition of 30 or 60 mg Zn/kg basal diet either from organic (zinc picolinate) or inorganic source (Z_nSO_4), with relatively higher improvement in birds supplemented with organic zinc source compared to inorganic source. Attia *et al.* (2015) reported that dietary supplementation of chromium, selenium and vitamin C separately or in combination decreased MAD concentration ($p<0.05$) in heat-tressed birds as compared with the control group. Rama Rao *et al.* (2016) found that the lipid

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	12.887 ± 1.51	12.825 ± 0.81	13.322 ± 1.16
T3	13.082 ± 1.66	13.778 ± 3.29	13.686 ± 1.70
T4	13.546 ± 1.48	13.925 ± 2.45	14.118 ± 1.24
T5	14.428 ± 0.93	13.947 ± 1.99	14.511 ± 0.90
T6	14.763 ± 0.53	14.413 ± 0.64	14.348 ± 1.66
T7	15.478 ± 2.20	14.477 ± 3.52	14.392 ± 1.10
T8	12.323 ± 2.33	11.437 ± 0.86	11.836 ± 1.05
T9	12.514 ± 2.10	12.465 ± 2.65	11.867 ± 0.96
T10	12.795 ± 0.49	13.457 ± 2.99	14.274 ± 1.43
T11	14.661 ± 0.54	14.071 ± 0.28	15.102 ± 1.52
T12	16.117 ± 0.61	17.178 ± 0.35	18.386 ± 3.47
T13	16.754 ± 2.05	17.421 ± 1.29	18.801 ± 3.20

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^{c} ± 4.43	8.566 ± 0.68	9.350^{e} ± 1.18
T2	13.672^{ab} ± 1.37	13.787 ± 2.38	14.295^{bcd} ± 0.74
T3	13.826^{ab} ± 1.61	14.625 ± 5.32	14.823^{bcd} ± 1.66
T4	15.983^{ab} ± 0.90	15.353 ± 6.91	16.265^{abc} ± 0.80
T5	12.431^{b} ± 1.58	12.349 ± 1.77	13.640^{bcde} ±1.61
T6	12.929^{b} ± 2.27	12.619 ± 2.96	13.604^{bcde} ± 1.81
T7	13.279^{b} ± 2.70	13.143 ± 1.24	14.486^{bcd} ± 1.28
T8	11.719^{bc} ± 3.52	9.129 ± 2.37	11.285^{de} ± 1.09
T9	12.257^{bc} ± 2.31	10.659 ± 0.57	12.373^{cde} ± 1.17
T10	12.377^{b} ± 1.59	11.390 ± 1.15	12.619^{bcde} ± 1.15
T11	13.797^{ab} ± 3.22	13.539 ± 2.24	13.572^{bcde} ± 2.68
T12	16.553^{ab} ± 0.16	16.953 ± 5.07	17.287^{ab} ± 2.76
T13	19.499^{a} ± 1.27	18.811 ± 2.68	20.230^{a} ± 1.66

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

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}± 0.01	3.36^{a}± 0.05	3.21^{a}± 0.01
T2	2.87^{b}± 0.09	2.69^{b}± 0.09	2.44^{b}± 0.03
T3	2.70^{c}± 0.05	2.59^{bc}± 0.05	2.38^{de}± 0.04
T4	2.54^{de}± 0.03	2.42^{de}± 0.03	2.29^{ef}± 0.02
T5	2.64^{cd}± 0.05	2.49^{cde}± 0..05	2.37^{de}± 0.03
T6	2.61^{cd}± 0.04	2.46^{de}± 0.04	2.35^{de}± 0.02
T7	2.62^{cd}± 0.03	2.41^{de}± 0.01	2.31^{ef}± 0.03
T8	2.68^{c}± 0.02	2.59^{bc}± 0.03	2.51^{b}± 0.02
T9	2.69^{c}± 0.02	2.58^{bc}± 0.04	2.42^{bc}± 0.04
T10	2.66^{c}± 0.02	2.47^{cde}± 0.02	2.37^{de}± 0.01
T11	2.59^{cde}± 0.02	2.59^{cd}± 0.03	2.54^{cd}± 0.04
T12	2.49^{e}± 0.01	2.38^{e}± 0.01	2.22^{fg}± 0.02
T13	2.36^{f}± 0.01	2.24^{f}± 0.03	2.15^{g}± 0.02

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

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	251.00 ± 14.29	238.67 ± 06.88	241.33 ± 11.46
T3	247.33 ± 31.88	228.67 ± 04.91	237.00 ± 06.35
T4	240.67 ± 16.37	225.67 ± 39.43	231.33 ± 07.05
T5	258.00 ± 15.87	239.67 ± 16.75	246.00 ± 06.08
T6	249.67 ± 20.95	234.00 ± 08.50	237.33 ± 06.93
T7	243.00 ± 33.60	227.00 ± 07.21	233.67 ± 04.17
T8	239.33 ± 04.84	223.33 ± 24.91	224.00 ± 34.50
T9	227.67 ± 08.17	215.67 ± 12.91	223.00 ± 16.50
T10	227.33 ± 22.74	212.00 ± 16.64	221.33 ± 11.46
T11	229.33 ± 22.92	221.67 ± 13.54	223.33 ± 13.24
T12	227.00 ± 05.50	212.00 ± 07.81	215.33 ± 13.86
T13	221.67 ± 12.58	207.33 ± 16.75	213.33 ± 11.83

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, Echeverry *et al.* (2016) studied that organic trace mineral supplementation showed no difference in plasma MDA level between control and OTM treatment (p>0.05) groups.

Biochemical parameters

Plasma glucose concentration

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 in organic chromium supplemented group of broilers (Table 6). 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. Tawfeek *et al.* (2014) reported that serum glucose level was significantly lowered in Cr; vitamins and Zn + Se groups which ranged from 80 to 87% as compared to control group (280 mg/dl).

Rosebrough and Steele (1981) 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.

Total protein, Albumin and Globulin concentration

The mean total plasma protein, albumin and globulin concentration showed significant difference (p<0.01).

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

Period / Treatment	21st day	28th day	35th day
T1	2.53^{e}± 0.02	2.53^{e}± 0.04	2.45^{f}± 0.08
T2	2.51^{e}± 0.07	2.47^{e}± 0.07	2.52^{f}± 0.06
T3	2.49^{e}± 0.11	2.54^{e}± 0.08	2.53^{f}± 0.04
T4	2.48^{e}± 0.06	2.52^{e}± 0.10	2.47^{f}± 0.06
T5	2.51^{e}± 0.08	2.49^{e}± 0.09	2.49^{f}± 0.05
T6	2.50^{e}± 0.11	2.45^{e}± 0.08	2.42^{f}± 0.13
T7	2.56^{e}± 0.12	2.53^{e}± 0.09	2.57^{f}± 0.05
T8	3.65^{d}± 0.09	3.64^{d}± 0.10	3.58^{e}± 0.13
T9	3.96^{c}± 0.06	3.96^{c}± 0.11	3.94^{d}± 0.08
T10	4.83^{b}± 0.19	4.83^{b}± 0.09	4.83^{b}± 0.19
T11	3.94^{cd}± 0.09	4.06^{c}± 0.05	4.03^{d}± 0.07
T12	4.15^{c}± 0.14	4.19^{c}± 0.13	4.40^{c}± 0.08
T13	5.28^{a}± 0.13	5.39^{a}± 0.12	5.35^{a}± 0.12

Means bearing different superscripts within same column 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^{d}± 0.03	1.64^{d}± 0.04	1.64^{d}±0.07
T2	1.63^{d}± 0.05	1.64^{d}± 0.03	1.65^{d}± 0.04
T3	1.63^{d}± 0.04	1.66^{d}± 0.05	1.63^{d}± 0.04
T4	1.62^{d}± 0.05	1.64^{d}± 0.05	1.65^{d}±0.05
T5	1.67^{d}± 0.04	1.69^{d}± 0.06	1.66^{d}± 0.08
T6	1.62^{d}± 0.02	1.64^{d}± 0.03	1.66^{d}± 0.04
T7	1.74^{d}± 0.04	1.66^{d}± 0.07	1.67^{d}± 0.05
T8	1.98^{c}± 0.09	1.94^{c}± 0.08	1.91^{c}± 0.02
T9	2.07^{abc}± 0.08	2.07^{bc}± 0.04	2.04^{bc}± 0.05
T10	2.14^{ab}± 0.06	2.16^{ab}± 0.03	2.15^{ab}± 0.06
T11	2.03^{bc}± 0.05	2.06^{bc}± 0.04	2.08^{bc}± 0.05
T12	2.17^{ab}± 0.04	2.23^{a}± 0.04	2.13^{b}± 0.08
T13	2.21^{a}± 0.03	2.24^{a}± 0.03	2.30^{a}± 0.07

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

between all the groups (Table 7, 8 and 9 respectively). Total plasma protein, albumin and globulin were higher for organic chromium supplemented groups.

The present findings were in agreement with Tawfeek *et al*. (2014) who reported that supplementation of Zn + Se and Cr significantly increased total protein levels in plasma. Attia *et al.* (2015) 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.

The probable reason behind increased serum concentration of total protein 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.

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

Period / Treatment	21st day	28th day	35th day
T1	$0.83^{e} \pm 0.01$	$0.86^{c} \pm 0.08$	$0.71^{ef} \pm 0.09$
T2	$0.83^{e} \pm 0.11$	$0.79^{c} \pm 0.05$	$0.83^{ef} \pm 0.04$
T3	$0.84^{e} \pm 0.15$	$0.84^{c} \pm 0.12$	$0.86^{e} \pm 0.09$
T4	$0.82^{e} \pm 0.06$	$0.84^{c} \pm 0.09$	$0.78^{ef} \pm 0.12$
T5	$0.80^{e} \pm 0.09$	$0.76^{c} \pm 0.04$	$0.78^{f} \pm 0.04$
T6	$0.85^{e} \pm 0.10$	$0.78^{c} \pm 0.05$	$0.70^{ef} \pm 0.12$
T7	$0.79^{e} \pm 0.12$	$0.84^{c} \pm 0.15$	$0.82^{ef} \pm 0.01$
T8	$1.59^{d} \pm 0.11$	$1.64^{b} \pm 0.05$	$1.59^{d} \pm 0.11$
T9	$1.82^{cd} \pm 0.11$	$1.83^{b} \pm 0.12$	$1.85^{cd} \pm 0.04$
T10	$2.64^{b} \pm 0.15$	$2.61^{a} \pm 0.11$	$2.59^{b} \pm 0.16$
T11	$1.85^{cd} \pm 0.13$	$1.71^{b} \pm 0.35$	$1.89^{cd} \pm 0.02$
T12	$1.94^{c} \pm 0.12$	$1.91^{b} \pm 0.17$	$2.16^{c} \pm 0.07$
T13	$3.01^{a} \pm 0.14$	$3.12^{a} \pm 0.09$	$2.96^{a} \pm 0.14$

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

Contrary to these findings Echeverry *et al*. (2016) 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.

Production performance parameters of broilers

Body weight

Significant difference (p<0.01) was observed in body weight gain between all the groups (Table 10). The maximum body weight gain (2141.25 ± 49.53 g) was attained in T13 group, supplemented with combination of organic form of Zn, Se and Cr followed by T7 group which were supplemented with organic form of selenium 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 Rama Rao *et al.* (2016), they reported that supplementation of Se, Cr and Zn as organic form significantly increased (p<0.05) body mass gain as compared to those fed the control diet. Among the trace minerals supplemented groups, the growth was significantly (p<0.05) higher in broilers fed with Se as compared to those fed Zn or Cr. Echeverry *et al.* (2016) 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 (Table 11). Maximum feed intake was observed for T13 (2.40 ± 0.09 kg) group and minimum feed intake was

Table 10: Mean body weight (g) of broilers birds at different intervals.

Period / Treatment	1st day	7th day	14th day	21st day	28th day	35th day	Body wt. gain
T1	46.75 ± 0.77	$108.12^{f} \pm 2.83$	$259.79^{h} \pm 12.26$	$502.45^{j} \pm 3.58$	$1152.54^{i} \pm 15.66$	$1450.04^{i} \pm 21.35$	$1403.29^{h} \pm 21.24$
T2	46.45 ± 0.69	$121.37^{e} \pm 4.28$	$344.16^{g} \pm 4.26$	$593.25^{i} \pm 7.37$	$1252.75^{h} \pm 14.96$	$1539.91^{h} \pm 23.91$	$1493.45^{g} \pm 23.69$
T3	46.33 ± 0.64	$154.08^{bcd} \pm 3.02$	$389.33^{cd} \pm 4.80$	$712.66^{h} \pm 10.03$	$1335.33^{g} \pm 13.63$	$1564.04^{gh} \pm 21.03$	$1515.66^{fg} \pm 21.04$
T4	46.75 ± 0.60	$161.83^{abc} \pm 2.63$	$478.25^{cd} \pm 10.41$	$818.83^{f} \pm 13.78$	$1447.41^{f} \pm 25.02$	$1705.04^{ef} \pm 22.57$	$1658.29^{e} \pm 22.52$
T5	46.75 ± 0.73	$154.91^{bcd} \pm 2.31$	$391.95^{f} \pm 3.21$	$760.66^{g} \pm 8.61$	$1360.50^{g} \pm 13.83$	$1629.45^{fg} \pm 20.24$	$1581.20^{f} \pm 20.37$
T6	46.79 ± 0.70	$156.04^{bcd} \pm 3.59$	$544.00^{b} \pm 4.01$	$980.12^{c} \pm 8.25$	$1529.62^{e} \pm 14.88$	$1832.62^{d} \pm 23.80$	$1785.75^{d} \pm 23.61$
T7	46.75 ± 0.60	$166.04^{a} \pm 3.18$	$776.66^{a} \pm 2.67$	$1104.00^{a} \pm 11.32$	$1747.79^{b} \pm 12.71$	$2068.58^{b} \pm 35.83$	$2021.45^{b} \pm 35.86$
T8	45.66 ± 0.67	$149.83^{d} \pm 2.41$	$332.50^{g} \pm 3.40$	$745.29^{gh} \pm 6.32$	$1357.75^{g} \pm 12.48$	$1526.12^{hi} \pm 24.40$	$1480.45^{g} \pm 24.12$
T9	46.37 ± 0.73	$155.20^{bcd} \pm 2.85$	$415.66^{e} \pm 2.40$	$867.70^{e} \pm 7.31$	$1612.08^{d} \pm 20.82$	$1730.83^{e} \pm 26.25$	$1684.45^{e} \pm 26.19$
T10	45.54 ± 0.74	$156.25^{bcd} \pm 3.00$	$488.79^{cd} \pm 3.50$	$968.83^{cd} \pm 6.67$	$1679.45^{c} \pm 13.62$	$1946.37^{c} \pm 24.87$	$1900.87^{c} \pm 25.11$
T11	45.83 ± 0.74	$154.12^{cd} \pm 4.30$	$473.66^{d} \pm 12.75$	$831.50^{ef} \pm 23.32$	$1438.25^{f} \pm 23.51$	$1616.83^{g} \pm 22.26$	$1573.50^{f} \pm 22.89$
T12	45.79 ± 0.69	$156.87^{bcd} \pm 3.87$	$492.20^{c} \pm 6.52$	$940.08^{d} \pm 22.50$	$1740.25^{b} \pm 22.62$	$1914.25^{c} \pm 30.83$	$1868.45^{c} \pm 30.77$
T13	45.87 ± 0.68	$163.41^{ab} \pm 2.89$	$477.12^{cd} \pm 10.21$	$1049.33^{b} \pm 26.85$	$1841.58^{a} \pm 26.95$	$2185.45^{a} \pm 49.22$	$2141.25^{a} \pm 49.53$

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

Table 11: Feed intake (g/bird/day) of broilers at weekly intervals.

Week / Treatment	1st	2nd	3rd	4th	5th	Total feed intake (Kg)
T1	20.22 ± 3.51	53.22 ± 3.43	69.16 ± 2.92	73.82 ± 5.43	92.31^{bc} ± 3.01	2.16^{bcd} ± 0.05
T2	20.92 ± 5.01	55.65 ± 3.61	70.25 ± 3.91	72.29 ± 2.82	93.27^{bc} ± 3.39	2.19^{bcd} ± 0.08
T3	18.21 ± 4.59	53.40 ± 2.92	67.32 ± 3.21	70.96 ± 4.20	86.30^{c} ± 2.33	2.07^{cd} ± 0.03
T4	20.26 ± 2.31	49.23 ± 2.94	64.93 ± 2.49	70.20 ± 3.12	87.24^{c} ± 3.43	2.04^{de} ± 0.01
T5	18.24 ± 0.66	50.79 ± 3.89	60.87 ± 3.17	63.32 ± 4.12	74.30^{d} ± 1.89	1.87^{e} ± 0.09
T6	19.16 ± 4.59	53.37 ± 3.98	68.14 ± 2.90	69.23 ± 2.61	85.32^{c} ± 2.36	2.06^{cd} ± 0.02
T7	24.18 ± 3.01	59.34 ± 2.81	73.17 ± 2.52	74.75 ± 4.10	89.23^{c} ± 2.96	2.24^{abc} ± 0.01
T8	18.16 ± 1.92	54.48 ± 2.31	67.31 ± 4.06	71.25 ± 3.55	88.28^{c} ± 3.61	2.09^{bcd} ± 0.07
T9	20.93 ± 2.74	54.78 ± 2.79	70.20 ± 2.62	72.77 ± 3.14	91.33^{c} ± 3.50	2.17^{bcd} ± 0.02
T10	22.19 ± 1.07	55.13 ± 2.53	68.25 ± 3.26	79.31 ± 2.26	100.95^{ab} ± 2.71	2.28^{ab} ± 0.02
T11	20.24 ± 1.91	53.12 ± 2.33	67.30 ± 4.16	70.10 ± 3.80	88.21^{c} ± 3.47	2.09^{cd} ± 0.08
T12	19.89 ± 1.20	56.14 ± 2.32	66.26 ± 4.52	72.20 ± 3.06	87.40^{c} ± 3.45	2.11^{bcd} ± 0.10
T13	22.32 ± 2.31	59.14 ± 3.41	73.31 ± 3.38	81.15 ± 2.94	106.33^{a} ± 1.62	2.40^{a} ± 0.09

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

Table 12: Production performance of broilers.

Parameter / Treatment	Wt. gain (g)	FI (g)	FER	PI	Mortality rate (%)
T1	1403.29^{h}± 21.24	2161.29^{bcd}± 52.22	0.65^{i}± 0.01	921.17^{f}± 28.09	00.00
T2	1493.45^{g}± 23.69	2186.84^{bcd}± 80.68	0.68^{hi}± 0.01	1030.47^{ef}± 32.85	00.00
T3	1515.66^{fg}± 21.04	2073.40^{cd}± 29.75	0.73^{fg}± 0.01	1117.39^{de}± 31.36	00.00
T4	1658.29^{e}± 22.52	2043.16^{de}± 8.38	0.81^{d}± 0.01	1360.39^{c}± 37.24	00.00
T5	1581.20^{f}± 20.37	1872.82^{e}± 96.26	0.84^{cd}± 0.01	1347.88^{c}± 35.21	00.00
T6	1785.75^{d}± 23.61	2066.68^{cd}± 23.02	0.86^{bc}± 0.01	1555.75^{b}± 40.69	00.00
T7	2021.45^{b}± 35.86	2244.78^{abc}± 11.22	0.90^{a}± 0.02	1843.20^{a}± 64.28	00.00
T8	1480.45^{g}± 24.12	2096.54^{bcd}± 75.66	0.70^{gh}± 0.01	1057.12^{e}± 33.80	04.17
T9	1684.45^{e}± 26.19	2170.21^{bcd}± 16.18	0.78^{e}± 0.01	1323.36^{c}± 40.56	04.17
T10	1900.87^{c}± 25.11	2280.99^{ab}± 16.21	0.83^{cd}±0.01	1594.65^{b}± 41.50	00.00
T11	1573.50^{f}± 22.89	2092.88^{cd}± 83.07	0.75^{ef}± 0.01	1192.69^{d}± 34.20	00.00
T12	1868.45^{c}± 30.77	2113.37^{bcd}± 101.97	0.88^{ab}± 0.01	1672.82^{b}± 54.07	00.00
T13	2141.25^{a}± 49.53	2395.82^{a}± 95.69	0.89^{ab}± 0.01	1944.44^{a}± 89.56	00.00

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

observed for T5 (1.87 ± 0.09 g) group. This might be due to synergistic effect 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 Rahaman *et al.* (2008), they reported that Ross broilers fed with zinc-methionine (40, 80 and 120 mg/kg) showed significantly higher feed intake as compared to inorganic source fed groups. Hamidi *et al*. (2016) found that improved cumulative weight gain was observed with diet supplemented with Chromium Picolinate in birds.

Feed efficiency ratio

FER differ also significantly (p<0.01) for all the treatment groups as compare to control group (Table 12). Maximum FER was observed for T7 (0.90 ± 0.02 g) followed by T13 (0.89 ± 0.01 g) and T12 (0.88 ± 0.01 g) group whereas, minimum FER was observed for T1 (0.65 ± 0.01 g) group.

Performance index

Significantly (p<0.01) maximum performance index was observed in T7 (1843.20 ± 64.28) and T13 (1944.44 ± 89.56) group whereas, minimum PI was observed for T1 (921.17 ± 28.09 g) and T2 (1030.47 ± 32.85 g) group (Table 12).

FER and PI were also significantly (p<0.01) different for the all treatment groups as compared to control group. Maximum FER and PI were observed in T7 followed by T13 and T12 group whereas, minimum FER and PI was observed for T1 group. This might be due to improvement in body weight gain and feed intake in broilers.

The present observations are in agreement with the findings of Krstic *et al*. (2012), they reported that increased feed efficiency and body weight gain in broilers diet supplemented with Se-enriched yeast.

Mortality

On perusal of data, it was observed that, mortality in broilers was found in only T8 (4.17%) and T9 (4.17%) group (Table 12).

Tawfeek *et al*. (2014) reported that supplementation of antioxidants improved the viability and decreased the mortality to the degree that no deaths occurred in the Zn + Se and Cr supplemented group. Echeverry *et al.* (2016) also reported that mortality in the OTM treatment group was 20% lower as compared to control.

CONCLUSION

Supplementation of organic form of Zn, Se and Cr (40, 0.30 and 2 mg/kg, respectively) both alone or in combination found to be necessary for glucose utilization in tissues and it also enhances protein synthesis, thus helps in faster body growth in broilers.

ACKNOWLEDGEMENT

The authors would like to acknowledge, M.P. Council of Science and Technology, Bhopal (M.P.) for providing financial assistance for the research project.

REFERENCES

Attia, kh. M., Tawfeek, F.A., Mady, M.S. and Assar, M.H. (2015). Effect of dietary chromium, selenium and vitamin con productive performance and some blood parameters of local strain dokki-4 under Egyptian summer conditions. Egyptian Poultry Science. 35(1): 311-329.

Bird, H.R. (1995). Performance index of growing chickens. Poultry Science. 34: 1163-1164.

Echeverry, H., Yitbarek A., Munyaka, P., Alizadeh M., Cleaver, A., Camelo-Jaimes, G., Wang, P.O.K. and Rodriguez-Lecompte J.C. (2016). Organic trace mineral supplementation enhances local and systemic innate immune responses and modulates oxidative stress in broiler chickens. Poultry Science. 95: 518-527.

Hamidi, O., Mohammad, C., Hasan, G., Ali, A.S. and Hassan, M.K. (2016). 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. 22(3): 373-380.

Krstic, B., Jokic, Z., Pavlovic, Z. and Zivkovic, D. (2012). Options for the production of selenized chicken meat. Biological Trace Element Research. 146: 68-72.

Rahman, J., Hasan, N.M. and Abbas, R. (2008). Improved broiler chick performance by dietary supplementation of organic zinc sources. Asian-Australasian Journal of Animal Science. 21(9): 1348-1354.

Rama Rao, S.V., Prakash, B., Raju, M.V.L.N., Panda A.K., Poonam, S. and Murthy, O.K. (2013). Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. Asian Australian Journal of Animal Science. 28(2): 247-262.

Rama Rao, S.V., Prakash, B., Raju, M.V.L.N., Panda A.K., Kumari, R.K. and Reddy, E.P.K. (2016). 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. 172(2): 511-520.

Rosebrough, R.W. and Steele, N.C. (1981) Effects of supplemental dietary chromium or nicotic acid on carbohydrate metabolism during basal, starvation and refeeding periods in poultry. Poultry Science. 60: 407-41.

Sahin, N., Sahin K., Onderci, M., Gursu, M. F., Cikim, G., Vijaya, J. and Kucuk, O. (2005). Chromium picolinate, rather than biotin, alleviates performance and metabolic parameters in heat stressed quail. British Poultry Science. 46: 457-463.

Snedecor, G.W. and Cochran, W.G. (1994). Statistical Method 8th Edn., The Iowa State University Press, Ames, Iowa, USA. pp 503.

Suri, K.P. (2015). Effect of organic zinc supplementation on performance, antioxidant status and immune response in white leghorn layers. M.V.Sc and A.H. thesis (Department of Poultry Science), Sri Venkateswara Veterinary University Rajendranagar, Hyderabad.

Tawfeek, S.S., Hassanin, K.M.A. and Youssef, I.M.I. (2014). The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. Journal of World's Poultry Research. 4(1): 10-19.