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The Effect of Dietary Antioxidants Supplementations on Growth Performance, Carcass Traits, and Immune Response of Heat-Stressed Broiler

Research Article

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ABSTRACT

The current study aimed to evaluate the effects of dietary antioxidants supplementations on growth performance, carcass traits, and immune response of heat-stressed broiler chickens. Four hundred chicks were randomly divided into five treatments (8 replicates of 10). By the end of day 21, the chicks were raised under normal temperature and from day 22 subjected to heat stress (37 degrees for five hours per day). Experimental treatments in the rearing period were 1: control (basal diet with normal levels of vitamins and minerals), 2: basal diet added vitamin E (100 mg/kg) and selenium (0.3 mg/kg), 3: basal diet added vitamin E (100 mg/kg), selenium (0.3 mg/kg) and vitamin C (300 mg/kg), 4: basal diet added vitamins E (100 mg/kg), C (300 mg/kg) and selenium (0.3 mg/kg), zinc (100 mg/kg), copper (10 mg/kg) and manganese (140 mg/kg) and the 5: basal diet added vitamins E (100 mg/kg), C (300 mg/kg) and selenium (0.3 mg/kg), zinc (100 mg/kg), copper (10 mg/kg), manganese (140 mg/kg) and chromium (1 mg/kg). The chelated minerals were used. Theses antioxidant supplementation resulted in improved weight gain, feed conversion ratio, and carcass traits (P<0.05). The effect of all supplements groups on the antibody titer against sheep red blood cells (SRBC) were significantly different compared with control (P<0.05). The blood parameters except thyroid hormones were affected by all supplements (P<0.05). The best treatment was the fifth treatment group which contained chromium. In conclusion, deleterious effects of heat stress on broiler performance can be effectively reduced by dietary antioxidants supplementation specially chelated trace minerals.

KEY WORDS antioxidant, broiler, chelated trace minerals, SRBC, vitamins.

INTRODUCTION

The adverse effects of high ambient temperature on viability, performance, and product quality are well described in poultry and continue to be economically detrimental on many farms. Heat stress (HS) causes deterioration in feed intake, weight gain, carcass traits, nutrient digestibility feed efficiency and carcass traits (Howlider and Rose, 1987; Sahine and Kucuk, 2003a). Moreover, HS was shown to adversely affect immune response against SRBC (Ghazi *et al*. 2012), as well as serum concentrations of minerals (e.g., Se, Zn, Cu, Mn, and Cr) and vitamins (e.g., vitamins A, E, and C), which are integral components of the antioxidant defense system (Sahine and Kucuk, 2003b; Sahin *et al.* 2009). One important harbinger of performance and physiologic decline in broilers is oxidative stress, the latter of which is a chief consequence of heat stress (Yang *et al.* 2010; Huang *et al*. 2015). Described as a disturbance in a biological system's defense against reactive oxygen species (ROS), heat-induced oxidative stress affects the capacity of several different antioxidant enzymes (Huang *et al*. 2015). Increases in ROS levels are harmful as they can lead to

DNA damage, protein denaturation, and lipid peroxidation (Huang *et al.* 2015). The enzymes glutathione peroxidase (containing selenium), superoxide dismutase (dependent on zinc, copper, and manganese), and catalase are the most important line of defense against peroxidants. As you can see in Figure 1, during heat stress, superoxide dismutase (SOD) is an important antioxidant as it is responsible for the catalysis of O_2^- into either simple molecular oxygen or the damaging compound hydrogen peroxide (H2O2) (Surai, 2015; Ighodaro and Akinloye, 2018).

In the case of H_2O_2 production, the complementary function of the antioxidant enzymes catalase and glutathione peroxidase 2 (GpX2) is required for detoxification (Dunning *et al.* 2013). Several methods are available to alleviate the negative effect of heat stress on birds and health status, such as dietary nutritional manipulation or feed additives (El-Kholy *et al.* 2017). In this respect, vitamins C, E, selenium, zinc, copper, manganese, and chromium are used in the poultry diet because of their anti-stress effects and also since their concentration is reduced during heat stress (Akbari and Torki, 2014; Habibian *et al.* 2014; Ghazi *et al.* 2015; Medeiros-Ventura *et al.* 2020; Hamidi *et al.* 2022). L-ascorbic acid (vitamin C) is an antioxidant vitamin and also has an important player in metabolic activity by declining as an electron donor (Khan *et al.* 2012). Many studies are investigating the effects of supplementation vitamin C to the diet to reduce or eliminate the negative effects of heat stress in chickens and also increasing feed intake, improving feed efficiency under heat stress (Büyükkılıç Beyzi *et al.* 2020). Another essential antioxidant is vitamin E (α-tocopherol), which helps removes free radicals and prevents lipid peroxidation (Rehman *et al.* 2017), and decreases plasma MDA level (Sahin and Kucuk, 2003b) in heat-stressed poultry. The main role or all metabolic functions in broiler chicks such as the secretion of the digestive system by improving nutrient digestibility is played by Trace minerals (Khan *et al.* 2012). Selenium (Se) is an antioxidant by the maintenance of tissue cellular integrity, which may affect growth performance in the broiler (Habibian *et al.* 2014). The function of Se is often augmented through interactions with vitamin E, which maximizes the efficiency of vitamin E as an antioxidant. Chromium (Cr) has been employed for feed efficiency to improve BW and relative organs in broiler chicks; therefore, Cr is considered a common mineral supplement, because it acts a dynamic role in enhancing the retention of other essential elements in blood and decreases their excretion (Sahin *et al.* 2017). Immunological function has been enhanced by Cr supplementation, and its effects seem more pronounced during times of acute heat stress. Both the organic and inorganic Cr supplements improved the immune response of broilers under heat-stress conditions, and positive effect was observed by addition of 1,200 ppb Cr-l-Met (Bahrami *et al.* 2012).

Zinc is known as one of the essential nutrients which is a cofactor for more than 200 enzymes in broiler chicks, also plays a central role in antioxidant capacity, growth, immunity, and digestion of nutrients by interaction with other minerals in the gut (Naz *et al.* 2016). Magnesium plays a role in more than 300 fundamental enzymatic reactions, such as the transfer of phosphate groups, the acylation of coenzyme A in the fatty acid oxidation process, the phosphate and pyrophosphate hydrolysis, amino acids activation, the synthesis and degradation of DNA, and has a key role in neurotransmission and immune function (Mario and Petracci, 2019). Copper (Cu) is a trace element that is necessary for many metabolic processes that occur inside the body (Scott *et al.* 2018), an essential component of various proteins and metalloenzymes, a critical element for iron metabolism, hemoglobin synthesis, and erythrocyte production. Cu has a fundamental role in the antioxidant defense system of the body through activation and being a part of antioxidant enzymes (Djoko *et al.* 2015). The present study explored the effect of dietary antioxidants supplementation (mix of vitamin (E and C) and chelated minerals (Se, Zn, Cu, Mn, and Cr) on growth performance, immune response, and blood metabolites of heat-stressed broiler and finally choosing the best antioxidant treatment in the face of heat stress.

MATERIALS AND METHODS

This study was conducted in agreement with the guidelines of the Ethics Committee of the Department of Animal Science, University of Zanjan, Zanjan, Iran (No. 15ZNU/05.12.2015). A total of 400 one-day-old male chicks, were generously provided by Gostaresh VA Tosse Behparvar and divided into 5 treatments. The chicks were randomly distributed into 40 experimental pens with 10 chicks per replicate (8 replicates per each treatment). Water and feed were provided *ad libitum* to the chicks. The diets (Table 1) were formulated and given according to Ross® nutrient specification for broilers (Aviagen, 2014). House temperature was maintained at 32 °C for the first 3 days and then gradually decreased to 23.9 °C at 21 days. The birds were raised under the 23L: 1D lighting program.

Growth performance

Data on feed intake (FI) and body weight (BW) were measured weekly, and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred and feed intake adjusted accordingly.

Table 1 Composition and chemical analysis of the broiler chicken diets (%)

Ingredients	Starter	Grower	Finisher
Corn (CP 8.8%)	53	57.8	63.17
Soybean meal (CP 44%)	40	35	30
Soybean oil	2	3	3
Di-calcium phosphate	2	1.7	1.5
Calcium carbonate	1	1	1
Salt	0.3	0.3	0.4
Sodium bicarbonate	0.1	0.1	0
DL-methionine	0.3	3	0.23
L-lysine hydrochloride	0.15	0.15	0.15
Vitamin and Mineral supplement[1]	0.5	0.6	0.5
Anticoccidial salinomycin	0	0.05	0.05
Chemical analysis			
Metabolizable energy (kcal/kg)	2890	3000	3070
Crude protein (%)	22.4	20.40	18.60
Calcium (%)	1	0.9	0.85
Available phosphorous (%)	0.5	0.44	0.4
Methionine (%)	0.63	0.61	0.49
Methionine + cysteine (%)	1.1	0.97	0.83
Lysine (%)	1.33	1.19	1.07
Arginine (%)	1.49	1.34	1.19

[1] Supplied per kilogram of diet: Retinol: 9000 IU; Alpha tocopherol acetate: 36 IU; Cholecalciferol: 2000 IU; Cyanocobalamin: 15 mg; Riboflavin: 6.6 mg; Calcium pantothenate: 9.8 mg; Niacin: 30 mg; Choline chloride: 625 mg; Biotin: 0.1 mg; Thiamine: 1.75 mg; Pyridoxine: 3 mg; Folic acid: 1 mg; Menadione: 2 mg; Antioxidant (ethoxyquin): 100 mg; Manganese: 248 mg; Zinc: 211 mg; Copper: 25 mg; Iron: 125 mg; Iodine: 2.5 mg and Selenium: 0.3 mg.

Temperature and treatments

The HS protocol was applied to the chicks from 22 until 42 days of age (Figure 1).

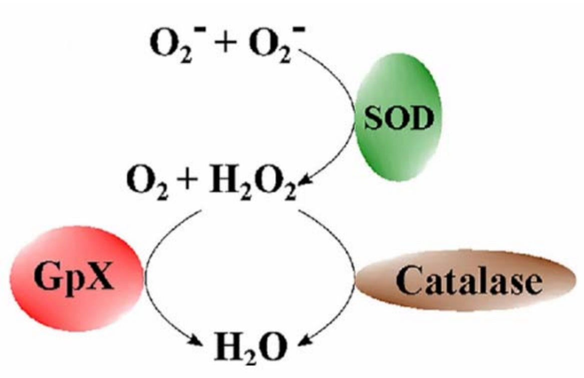

Figure 1 During heat stress, the production of superoxide radicals increases, he latter of which are converted by SOD to hydrogen peroxide and oxygen. Hydrogen peroxide is then detoxified either by GpX or catalase to produce water

The birds were daily exposed to 23.9 °C for 12 h, 23.9 to 37 °C for 4 h, 37 °C for 5 h, and 37 to 23.9 °C for 3 h (Habibian *et al*. 2014). From the 22 days, at the same time with the onset of HS, birds were assigned to five groups of vitamin and chelate-antioxidant mineral (Zinpro Corporation) supplements as on top. Experimental treatments are shown in Table 2.

Table 2 Experimental treatments

Treatment	
1	Control
2	Vitamin E (100 mg/kg) + Se (0.3 mg/kg)
3	Vitamin E (100 mg/kg) + Se (0.3 mg/kg) + Vitamin C (300 mg/kg)
4	Vitamin E (100 mg/kg) + Se (0.3 mg/kg) + Vitamin C (300mg/kg) + Zn (100 mg/kg) + Cu (10 mg/kg) + Mn (140 mg/kg)
5	Vitamin E (100mg/kg) + Se (0.3 mg/kg) + Vitamin C (300 mg/kg) + Zn (100 mg/kg) + Cu (10 mg/kg) + Mn (140 mg/kg) + Cr (1 mg/kg)

Sheep red blood cells (SRBC) antibodies

On day 21, two chicks per pen were marked and injected in the right-wing vein with 1 mL 7 % suspension of SRBC. Ten days after injection (days 32), blood samples were obtained from marked birds by left-wing venipuncture 10 h after an overnight feed deprivation, and then 3 ml blood was collected.

The samples were incubated at 37 °C for 1 h to aid clotting and retraction then centrifuged at 1,500 g for 10 min and the serum was collected and stored at −20 °C until the hem agglutination assay to analyze the antibody response to SRBC. Serum samples were tested for IgM and IgG using the 2-mercaptoethanol (ME) technique as previously described (Ohkawa *et al*. 1979; Qureshi and Havenstein, 1994).

Blood metabolites

At 42 days of age, 2 birds from each pen were randomly selected (each selected bird was around the average body weight for the group), weighed, and bled by wing vein puncture.

Non-heparinized collected blood samples were centrifuged for 20 min at 3000 rpm. Obtained serum was frozen and stored at −20 °C until further analysis. Serum glucose, triglycerides, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total cholesterol, and total protein were analyzed using the diagnostic kit (Pars Azmun, Iran), and enzymatic methods. The plasma concentrations of T4 and T3 were measured by using the ELISA Kit (IDS Ltd, Boldon, UK) and commercial ELISA kit (Pishtaz-Teb, Tehran, Iran) according to the manufacturer's recommendations. Lipid peroxidation was assayed calorimetrically as a 2- thiobarbituric acid reactive substance (TBARS) using the modified method of Ohkawa *et al.* (1979) described by Mujahid and Neil (2007). The TBARS content was assayed by using a spectrophotometer (Hitachi U-2001, Schaumburg, IL) at 532 nm and expressed as Nano-moles of MDA per ml sample.

Carcass traits

At the end of the experiment (day 42), 2 Chickens from each replicate were slaughtered, and then their organs were weighted individually. Liver, lymphoid organs (spleen and bursa of Fabricius), heart, breast, thighs, different parts of the small intestine, and abdominal fat were measured and expressed as a percentage of the live body weight.

Statistical analysis

The experimental design was a completely randomized design with 5 treatments. The experimental unit differed according to the parameter measured. For performance characteristics, the experimental unit was a pen, whereas individual chick data were used for immunological, carcass traits, and blood parameters. All data were analyzed using the general linear model (GLM) of the SAS (2003), and differences among treatment means were determined using Duncan's multiple range test (P≤0.05). Means were compared using Duncan's multiple range test. The statistical design used was as follows:

$$Y_{ijk} = \mu + Ai + Eajk + Bj + A \times Bij + Ebijk$$

Where:
Yijk: observation related to treatment i and measurement time j in repetition k.
μ: overall average of observations.
Ai: effect of treatment.
Eaik: main error (effect of treatment i in repetition k).
Bj: effect of period.
ABij: interaction effect of treatment i in period j.
Ebijk: secondary error.

RESULTS AND DISCUSSION

The effect of experimental treatments on growth performance in the period of 22 to 42 days is shown in Table 3. The results showed that the addition of antioxidant supplements to the diet had a significant effect on final body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR), (P<0.01). Table 2 and Figures 2 and 3 show, broilers fed with antioxidant supplements E, C, Se, Cu, Mn, Zn, Cr had the highest final body weight, body weight gain, and lowest feed conversion ratio (P<0.05). The lowest final body weight, body weight gain, and highest feed conversion ratio were related to E, Se supplementation.

Table 3 Effect of antioxidant supplementation on performance of heat stressed broiler on day 42

Treatment	BW	BWG	FI	FCR
Control	2093[bc]	1237[bc]	3298	2.67[b]
E , Se	2019[c]	1163[c]	3340	2.86[a]
E, C, Se	2111[bc]	1255[bc]	3308	2.63[b]
E, C, Se, Zn, Cu, Mn	2243[b]	1387[b]	3294	2.37[c]
E, C, Se, Zn, Cu, Mn, Cr	2452[a]	1596[a]	3283	2.06[d]
SEM	50.1	44.2	65.8	0.08
P-value	<0.0001	0.001	0.54	<0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

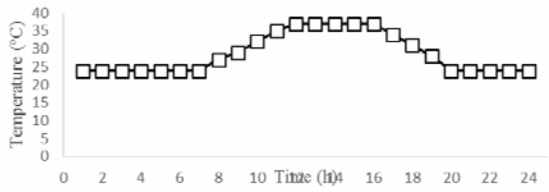

Figure 2 Heat stress patterns applied to broiler chicks in this experiment (3–5 weeks of age). Cyclic heat stress (23.9–37 °C) was applied

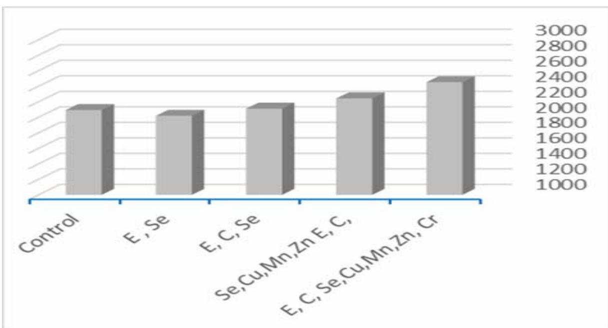

Figure 3 Effect of antioxidant supplementation on body weight of heat stressed broiler 42 d

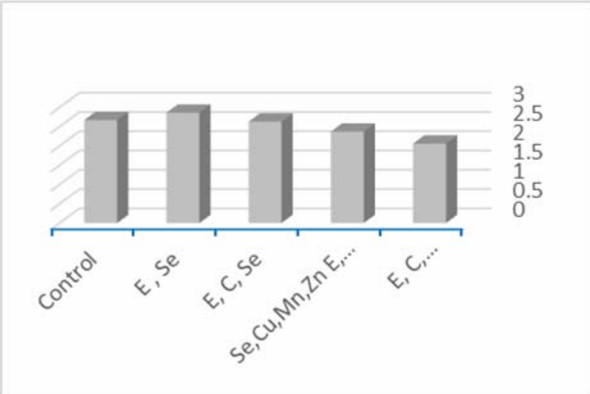

Figure 4 Effect of antioxidant supplementation on FCR of heat stressed broiler 42

The results for the effects of adding antioxidant supplements to the diet on carcass traits and visceral indices are given in Tables 4 and 5. The results showed that antioxidant supplements had a significant effect on carcass percentage, abdominal fat percentage, heart percentage, and bursa percentage (P<0.05), and the effect of these supplements on spleen percentage tended to be significant. The effect of supplements on the percentage of the duodenum was significant (P<0.05) and the cecum tended to be significant.

Except for vitamin E and Se supplements, other antioxidant supplements significantly increased carcass percentage. Also, the percentage of thighs in the supplements had a decreasing trend compared to the control treatment. The percentage of abdominal fat in E, C, Se, Cu, Mn, Zn, and Cr supplements showed a significant decrease compared to the control treatment and other supplements treatments had intermediate numbers.

Except for vitamin E and Se treatment, in other supplements, the percentage of heart was significantly reduced compared to the control group. The percentage of duodenum and bursa was the highest in the control treatment and supplements reduced them.

The addition of antioxidant supplements (P<0.05) on the antibody titer against SRBC was significant. The highest amount of immunoglobulins was observed in the supplements treatment (E, C, Se, Cu, Mn, Zn, and Cr), which was significantly different from other supplements and control treatment (Table 6).

The effect of treatments on blood parameters is shown in Tables 7 and 8. The results showed that all blood parameters except thyroid hormones were affected by antioxidant supplements (P<0.05). According to the results, vitamin E and Se and its combination in other treatments had similar effects on HDL concentration and increased its level compared to the control group.

However, the combination of vitamin E and Se in other treatments could have better effects on HDL concentration. The results showed that the addition of vitamin E and Se group had a better effect on LDL, compared to the combination with other antioxidants. Triglyceride and malondialdehyde (MDA) concentrations were significantly lower in all supplement treatments compared to the control group.

The addition of vitamin E and Se did not affect glucose concentration, but in combination with other vitamin and minerals, the glucose concentration decreased, which is probably related to other supplements. The lowest glucose concentrations were observed in E, C, Se, Cu, Mn, Zn, and Cr treatment. Total protein concentration in all antioxidant supplement groups was significantly higher than the control treatment.

There are several reports with conflicting results about the effect of vitamin E and Se on feed intake in different environmental conditions. For example, while extensive research has shown that vitamin E does not affect feed intake (Niu *et al.* 2009; Vakili *et al.* 2010), others reported that the use of high levels of vitamin E in the diet of heat-stressed broilers increased feed intake (Habibian *et al.* 2014).

These researchers stated in justifying their results that one of the main functions of vitamin E is to prevent lipid peroxidation, especially the unsaturated fatty acids that make up cell membranes. The researchers also reported that selenium is also an antioxidant and through its antioxidant activity maintains cell shape and integrity, thereby causing cells to be affected by stress during heat stress (Habibian *et al.* 2014). Consistent with the findings of the present study, Peric *et al.* (2019) reported that the use of selenium supplementation has no effect on the amount of feed intake by broilers.

Ghazi *et al.* (2015) in a study on broilers under heat stress found that the use of vitamin C supplements in the diet led to improved performance at 42 days of age. The improvement in the performance of vitamin E, selenium and vitamin C compared to vitamin E and selenium treatment is probably due to the effect of adding vitamin C. Accordingly, Peˇcjak *et al.* (2022) reported that vitamin E and vitamin C have synergistic effects in reducing the negative effects of heat stress in broilers. Vitamin E is the most important antioxidant in the cytoplasmic membrane and vitamin C is the most important antioxidant in the cytoplasm and aquatic spaces. In line with the results of this study, the Saiz Del Barrio *et al.* (2019) studied the effect of adding vitamin C and mineral complexes on broilers under heat stress; the mineral complex has led to improved growth performance. At higher or lower temperatures than the bird's temperature comfort zone, corticosteroid secretion increases in response to stress. Kutlu and Forbes (1993) stated that ascorbic acid supplementation reduces the synthesis of corticosteroid hormones in birds under heat stress.

Table 4 Effect of antioxidant supplementation on carcass trait (expressed as percentage of the live body weight) of heat stressed broiler 42 d

Treatment	Carcass	Breast	Thigh	Fat	Heart	Spleen	Bursa
Control	62.80[b]	27.07	20.14	1.15[a]	0.56[a]	0.145	0.179[a]
E , Se	62.77[b]	27.08	18.79	1.20[a]	0.51[ab]	0.148	0.138[b]
E, C, Se	63.52[a]	26.95	17.94	1.34[a]	0.46[b]	0.171	0.135[b]
E, C, Se, Zn, Cu, Mn	63.63[a]	26.91	18.44	1.03[ab]	0.47[b]	0.141	0.145[ab]
E, C, Se, Zn, Cu, Mn, Cr	63.37[a]	28.01	18.37	0.76[b]	0.45[b]	0.121	0.137[b]
SEM	0.13	0.72	0.65	0.09	0.02	0.10	0.012
P-value	0.001	0.866	0.09	0.003	0.001		0.05

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of antioxidant supplementation on visceral trait(expressed as percentage of the live body weight) of heat stressed broiler

Treatment	Liver	Ileum	Duodenum	Jejunum	Cecum
Control	2.68	1.32	1.01[a]	1.47	0.819
E , Se	2.51	1.16	0.856[b]	1.26	0.677
E, C, Se	2.45	1.23	0.818[b]	1.39	0.665
E, C, Se, Zn, Cu, Mn	2.44	1.21	0.888[b]	1.35	0.668
E, C, Se, Zn, Cu, Mn, Cr	2.29	1.20	0.79[b]	1.33	0.730
SEM	0.07	0.07	0.03	0.07	0.04
P-value	0.688	0.720	0.031	0.489	0.076

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of antioxidant supplementation on SRBC of heat stressed broiler

Treatment	IgG	IgM
Control	2.04[c]	0.57[b]
E , Se	2.26[b]	0.72[a]
E, C, Se	2.27[b]	0.70[a]
E, C, Se, Zn, Cu, Mn	2.29[b]	0.70[a]
E, C, Se, Zn, Cu, Mn, Cr	2.39[a]	0.71[a]
SEM	0.02	0.009
P-value	<0.0001	<0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

By reducing the synthesis and secretion of corticosteroids by supplementing the diet with vitamin C, the negative effects of stress on poultry performance are reduced. Because environmental stress is one of the causes of oxidative stress that reduces the concentration of antioxidant vitamins in plasma such as vitamins A, E and C and increases the excretion of minerals such as zinc, manganese, copper, selenium, chromium and iron, which these stresses lead to increased oxidative damage in the bird.

On the other hand, these stresses lead to increased production of free radicals and lower concentrations of antioxidant vitamins and minerals in serum and tissues (Sahin and Kucuk, 2003b). Zinc and manganese are essential for the normal functioning of many of the structural proteins, enzymes, and cellular proteins needed for the growth and development of broilers. In accordance with the results of the present experiment, Medeiros-Ventura *et al*. (2020) reported that the addition of zinc-manganese-copper organic chelate to the diet of laying hens under cold stress has improved the productive performance of birds. The researchers attributed the improvement in performance due to the consumption of organic mineral complex to the use of zinc in improving the quality of intestinal epithelial tissue, increasing nutrient uptake. Chromium and zinc are antioxidants; they have a protective role for pancreatic tissue against oxidative damage caused by free radicals, which may affect pancreatic function by increasing digestive enzymes and improving Nutrients digestibility. Akbari and Torki (2014) showed also that the addition of chromium to the diet of broilers could improve the body weight of broilers under heat stress. Explaining their findings, the researchers said that adding chromium to the diet under heat stress increased insulin concentration and glucose uptake, and improved live weight and feed efficiency. The researchers also said that stressors such as heat stress lead to the release of chromium from tissues and their excretion, and therefore may exacerbate chromium deficiency and increase chromium requirements. Contrary to the findings of the present study, Biswas *et al*. (2006) reported that the use of 0.5 mg/kg selenium in the diet of Japanese quails had no effect on the weight of liver and spleen. Bartlett and Smith (2003), Niu *et al*. (2009) and Habibian *et al*. (2014) reported that exposure to heat stress reduces the relative weight of lymphatic and liver organs in broilers. The researchers suggested that weight loss in the lymphatic organs may be the result of reduced feed intake, as the amount of nutrients needed to develop these organs decreases as feed intake decreases. In addition, the oxidative stress caused by exposure to heat stress increases lipid peroxidation, which may be another reason for the reduction of these organs (Pamok *et al*. 2009).

Table 7 Effect of antioxidant supplementation on blood parameters of heat stressed broiler

Treatment	HDL	LDL	Triglyceride	Cholesterol	Glucose	Total Protein
Control	30.31[c]	46.87[a]	120.62[a]	124.06[a]	207.37[b]	3.13[b]
E , Se	33.18[b]	42.50[b]	111.87[b]	112.62[c]	235.50[a]	3.45[a]
E, C, Se	34.43[b]	46.18[a]	110.43[b]	120.00[ab]	201.25[b]	3.40[a]
E, C, Se, Zn, Cu, Mn	37.21[a]	45.57[a]	111.00[b]	118.14[b]	194.78[c]	3.51[a]
E, C, Se, Zn, Cu, Mn, Cr	36.00[a]	43.92[b]	109.92[b]	119.71[ab]	194.35[c]	3.54[a]
SEM	0.453	0.497	1.117	1.470	1.88	0.049
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of antioxidant supplementation on thyroid hormone and malondialdehyde (MDA) of heat stressed broiler

Treatment	T_3 (ng/mL)	T_4 (ng/mL)	MDA
Control	1.28	9.26	2.60[a]
E , Se	1.25	9.31	2.23[b]
E, C, Se	1.29	9.28	2.23[b]
E, C, Se, Zn, Cu, Mn	1.29	9.27	2.25[b]
E, C, Se, Zn, Cu, Mn, Cr	1.25	9.31	2.15[b]
SEM	0.027	0.037	0.034
P-value	0.663	0.0808	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results of the present study showed that the feed intake was not affected by additives that affect the weight of these organs. Heat stress prevents the absorption of nutrients, which affects the weight of the organs. Contrary to the findings of our study, also Hesabi Nameghi *et al.* (2007) reported that chickens receiving 50 mg/kg of vitamin E had the highest weight of thymus and spleen and the use of higher levels of vitamin E had no effect on the weight of these organs. They also reported that the use of diets containing 75 mg/kg of vitamin E reduced the weight of the Fabricius bursa. It is suggested that providing diets containing high concentrations of essential nutrients for broiler chickens maintains the level of consumption of these nutrients and thus can improve immune responses in stress conditions.

Such a result was observed in the present study, the addition of micronutrients could improve immune responses, which is probably related to the synergistic effects between micronutrients for antibody properties. Consistent with the findings of the present study, Habibian *et al.* (2014) reported that elevated levels of vitamin E up to 50 mg/kg improved the antibody titer against SRBC. They examined the effect of different levels of vitamin E and selenium on the humoral immune response of broilers. In the present study, the effects of vitamin E and selenium were similar to their effects in combination with other treatment expect chromium.

These results show that the effects of vitamin E and selenium with other minerals and vitamin C are only related to vitamin E and selenium antioxidant, and other compounds have not had many synergistic effects with it. However, the combined effects of chromium led to better effects of vitamin E and selenium and improved immune responses under stress.

Another study has reported improvements in the antibody response and the immune system with zinc-copper-manganese supplementation (Medeiros-Ventura *et al.* 2020). Deficiencies of these elements are effective in reducing immunity, so that zinc deficiency leads to a decrease in cellular immunity, development of thymus and spleen and reduced production of interleukin. The absolute number and relative percentage of T cells, especially T-helper cells, is reduced by copper deficiency and significantly reduces the antibody response by increasing susceptibility to infections. When chickens are exposed to high ambient temperatures, heat stress causes lipid peroxidation to increase, resulting in the production of more free radicals. Increased production of lipid peroxidation increases the concentration of lipids in the blood (Olfati *et al.* 2018). Under such conditions, the addition of antioxidants to the diet reduces their concentration in the blood. Kanchana and Jeyanthi (2010) observed that the use of different levels of vitamin E or selenium increases the concentration of HDL-cholesterol and decreases the concentration of LDL-cholesterol in laying hens. Facing such conditions causes the body to lose its antioxidant supply and the animal is deficient in nutrients such as vitamin E and selenium, copper, zinc, chromium, vitamin C, etc. Increased HMG-CoA reductase activity seems to be the most important factor in increasing cholesterol and LDL concentrations and decreasing HDL concentrations in selenium deficient mice (Qu *et al.* 2010). Akbarzadeh *et al.* (2008) also showed a decrease in the concentration of cholesterol and triglycerides in rats following the use of selenium supplementation as a result of a possible increase in the activity of the phospholipid hydroperoxide glutathione peroxidase in the reduction of hydroperoxides of fatty acids and L-cholesterol in the membrane.

However, the main effect of vitamin E in lowering cholesterol and triglycerides is attributed to its transfer by LDL. But another important factor that may affect the oxidation of LDL and therefore the concentration of lipids is the amount of HDL in the environment. In fact, HDL acts as the main carrier of antioxidants in the bloodstream and also prevents LDL oxidation in different ways. It also appears that HDL can replace healthy phospholipids with damaged phospholipids in LDL. Thus, transfer of alpha-tocopherol by HDL can increase the antioxidant effect of HDL (Habibian *et al.* 2014).

Regarding the effect of chromium on lipids, it should be noted that chromium is an essential element involved in the metabolism of carbohydrates, lipids and proteins. Numerous studies have shown that chromium is an important factor in cholesterol synthesis and its reduction in farm animals. Trivalent chromium plays an important role as a component of glucose tolerance factor as it enhances the effects of insulin and leads to the regulation of lipid metabolism and the use of cholesterol. At low levels of insulin, cholesterol is converted to fat and stored in fat cells (Akbari and Torki, 2014). Chromium stimulates the conversion of acetate to CO_2, cholesterol and fatty acids. High levels of chromium are needed to stimulate cholesterol synthesis in the liver (Uyanik, 2001). A very simple explanation for the hypolipidemic effects of chromium is associated with increased glucose tolerance and decreased lipolysis. Chromium increases the efficiency of insulin, which insulin also reduces the concentration of Ketone bodies in the blood in three related ways: 1-Reduces the breakdown of fats, which reduces the supply of non-esterified fatty acids to the liver. 2- Reduces the production of Ketone bodies.3- Facilitates the use of Ketone bodies (Akbari and Torki, 2014).

In relation to copper and vitamin C, Idowu *et al.* (2011) in a study examined the effect of using 250 mg/kg levels of copper supplement and 100 mg/kg of ascorbic acid on broiler chickens. HDL-cholesterol, LDL-cholesterol and total cholesterol levels compared to the control group Decreased. Exposure to heat stress is one of the most important factors that increase the concentration of malondialdehyde in the muscle and other tissues of broilers. The results of this study also showed an increase in the concentration of malondialdehyde under heat stress conditions.

Chromium in the diet may be needed to maintain the normal sensitivity of pancreatic beta cells to glucose, or to produce insulin. It has been shown in rats that chromium is required to maintain the normal sensitivity of pancreatic beta cells to prevent high insulin secretion. Akbari and Torki (2014) collectively stated that chromium is a cofactor for insulin activity and is necessary for normal use of glucose and animal growth and is essential for normal glucose metabolism and is a component of glucose tolerance factor (GTF), which through insulin, it enters glucose into the cell and produces energy. Insulin regulates the metabolism of carbohydrates, proteins and also the use of glucose. Vakili *et al.* (2010) reported that exposure of broilers to heat stress conditions increased their serum glucose concentration, and vitamin E supplementation decreased glucose levels only in such conditions. Consistent with the results of our study, Habibian *et al.* (2014) showed that the addition of vitamin E and selenium to the diet had no significant effect on blood glucose. Overall, the results of this section show that stress raises blood glucose and that a glucose tolerance factor such as chromium needs to be added to the diet to lower blood glucose. In our experiment total protein content was lower in control treatments and additives increased it. Increasing corticosteroid levels when exposed to heat stress conditions reduces protein synthesis. Thus, the destruction of lymph cells following a decrease in protein synthesis reduces the production of antibodies.

One of the most important effects of corticosterone is an increase in glucose production during muscle protein catabolism. This issue increases uric acid production in addition to increasing glucose production in the gluconeogenesis pathway (Habibian *et al.* 2014). However, as previously mentioned chromium affects insulin and increases the concentration of corticosterone under heat stress, which inhibits the absorption of glucose and amino acids, in which case chromium, by affecting insulin RNA, causes Protein and glucose, thus inhibiting protein catabolism and uric acid production and heat stress conditions reduce protein concentration.

CONCLUSION

In conclusion, deleterious effects of heat stress on broiler performance can be effectively reduced by dietary antioxidants supplementations. The effects of vitamin E and selenium combined with other minerals and vitamin C are only related to vitamin E and selenium antioxidant, while the combined effects with chromium led to better effects of vitamin E and selenium and improved the responses under heat stress.

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