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Original Article

The Effect of Dietary Supplementation of Some Antioxidants on Performance, Oxidative Stress, and Blood Parameters in Broilers under Natural Summer Conditions

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ABSTRACT

The aim of the study was to evaluate the effects of dietary supplementation with some antioxidants (vitamins & minerals) against the deleterious impacts of heat stress on broilers. One hundred and twenty eight broiler chicks were randomly assigned into 4 dietary groups with 4 replicates (8 chicks each). The chicks were raised for 6 weeks under natural summer months (36 °C and 75% RH) during which the birds received either a basal diet only (control group), or the basal diet supplemented with either 250 mg vitamin C+ 250 mg vitamin E/ kg diet, or organic microminerals: 40 mg Zn + 0.30 mg Se/ kg diet, or with 0.50 mg Cr/kg diet. Another group of birds which consists of 32 broiler chicks (4 replicates, 8 birds each) was reared under natural autumn conditions (25 C° and 67% RH) and fed on the same control diet. This group was used to stand out the effects of heat stress per se through comparing its results with those of the previous control one. It was found that high ambient temperature severely reduced body weight, feed intake, and feed efficiency as well as increased abdominal fat and mortality rate. However the supplementation of antioxidants was able to alleviate many of these effects. The heat stress condition significantly increased serum cholesterol, glucose, and malondialdehyde and decreased protein and glutathione peroxidase. Even though, the used supplements improved the blood profile parameters and the oxidative status of birds. The present results indicate that the supplementation of diets with antioxidants, especially vitamins and chromium, is necessary to overcome the deleterious effects of heat stress on broilers' performance.

Keywords: Broilers, Heat Stress, Vitamin C, Vitamin E, Zinc, Selenium, Chromium

INTRODUCTION

Recently, climate changes and increasing temperatures in various regions, and/or increasing extremities in weather patterns have been shown. In Egypt ambient temperature can remain consistently high for extended periods of time in addition to sudden recurrent hot and humid waves which have more harmful effect. So poultry production suffers significant losses every year because of heat stress, leading to economic losses to the poultry farmers. During these periods temperatures approached 40˚C most of the time and humidity reach 75%. A temperature above 30˚C represents a heat- stressed condition for birds and is one of the most common stressors that affect the production criteria in poultry where the ideal temperature for broilers is 10-22˚C to get optimum body weight and 15-27˚C for feed efficiency (Rama Rao et al., 2011).

Heat stress is principally important in intensive poultry operations especially in broilers lines because their higher production performance and feed conversion efficiency make today's chickens more susceptible to heat stress than even before (Lin et al., 2005). High mortality, decreased feed intake, lower body weight gain and poor feed efficiency are common adverse effects of heat stress often seen in meat-type poultry flocks (Yegani, 2008). In addition, heat stress increases lipid oxidation as a consequence of increased free radical generation, a condition that enhances the formation of reactive oxygen species (ROS) and induces oxidative stress in cells. Antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) play a vital role in protecting cells from harmful effects of ROS (Altan et al., 2003). Synthesizing these enzymes is an important regulation, in terms of animal response to stress conditions. However, this response will be effective only if cofactors such as Se for GPx and copper, zinc, and manganese for SOD are available (Underwood, 1977; McDowell, 1989).

Reducing stress in poultry remains a topic of concern amongst producers and scientists. Several

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methods are available to alleviate the negative effects of high environmental temperature on performance of poultry. Because of the high cost and impractical of cooling animal buildings, such methods are focused mostly on the dietary manipulation (Konca et al., 2009). Vitamin E is known to be a protective of lipid component of biological membranes as it is considered a major chain-breaking antioxidant (Halliwell and Gutteridge, 1999).Vitamins C and E present important metabolic interactions where vitamin C enhances vitamin E antioxidant activity by reducing tocopheroxyl radicals into the active form of vitamin E (Jacob, 1995), or sparing available vitamin E (Retsky and Frei, 1995). Also vitamin E and Se act together in preventing cellular membranes from oxidative degeneration (Hoekstra, 1975; Combs and Scott, 1977). GPx contains Se as a constituent and has been proposed as an index for assessing the Se status. Combs and Pesti (1976) showed that the ascorbic acid increased GPx activity in plasma and reduced the dietary Se requirement of vitamin E- deficient chicks. Studies showed that concentrations of malondialdehyde (MDA), an indicator of lipid peroxidation, in serum and liver decreased with Zn supplementation in heat- stressed birds. In addition, Zn supplementation significantly increased serum concentrations of vitamins C & E and Zn in poultry (Sahin and Kucuk, 2003). One of the most important functions of Zn is related to its participation in the antioxidant defense system. Dietary chromium supplementation increases the growth rate and feed efficiency and improves meat yield and carcass quality with reduced carcass fat in broilers (Toghyani et al., 2006; Samanta et al., 2008). Its beneficial effects appear to be greater under stress (Borgs and Mallard, 1998).

In this study the effect of dietary supplementation with some antioxidant nutrients either vitamins (C+E) or organic microminerals (Zn+Se) and trivalent Cr on the growth performance, carcass, blood parameters and antioxidant status in broilers under heat stress was evaluated. Furthermore, the present study investigated the effects of heat stress per se on the birds by comparing them with those reared under thermoneutral conditions.

MATERIALS AND METHODS

Birds

One hundred and twenty eight, one day-old (Cobb-500) broilers, after elimination of obvious runts and chicks in extreme weights, provided by a commercial company (Elshourok company, Egypt) were used in this study which lasted up to 42 days of bird's age in natural environments during Egyptian summer months from 16 July to 26 August . The birds were assigned to 4 experimental groups (heat- stressed control and 4 supplemented groups) with 4 replicates and 8 birds per pen. All pens were bedded with a wheat straw litter and equipped with feed and water utensils.

Diets and experimental design

Eight experimental diets, 4 in each feeding period (starting/growing), were formulated according to the requirements suggested by the NRC (1994) guidelines. Control diets (basal diets without the tested supplements) contained 23% and 20% protein for starter and grower diets, respectively with 3200 kcal of ME/kg in both. Ingredients and chemical composition of the control diets are shown in Table 1. Ingredients were analyzed for their proximate composition using the standard methods according to AOAC (1995) and the diets were formulated based on these values. Each kilogram of the control diet contained in addition to that found naturally in feedstuffs 20 mg vitamin E and 56.81mg inorganic Zn and 0.107mg inorganic Se (derived from the added vitamin – mineral premix). The premix did not contain vitamin C or chromium.

The other three experimental diets were formulated to study the effect of the additional allowances of antioxidant nutrients. These diets were formulated by supplementation of the basal diet either with vitamins; 250 mg/kg vitamin E (as α- dl tocopherol acetate, Roche, Egypt) and 250 mg/kg vitamin C (L- ascorbic acid, Roche, Egypt) in vitamins group, or with organic trace minerals; 40 mg/kg Zn (as zinc chelated with methionine hydroxyl analogue, Zn-MET/10, IBEX international Co.) and 0.30 mg/kg Se (as Se enriched yeast, Sel- Plex, Alltech Inc.) in Zn+Se group; or with 0.50 mg/kg Cr (as chromium picolinate (CrPic), Engromix Com.) in Cr group.

Table 1. Ingredients and chemical composition of the control diets

Ingredients, %	**Starter**	**Grower**
Yellow corn	49.34	57.51
Soybean meal	30.35	27.85
Corn gluten	10.00	6.00
Sunflower oil	5.89	5.16
Calcium phosphate, basic	1.75	1.23
Limestone powder	1.71	1.49
Sodium chloride	0.49	0.35
DL-methionine	0.08	0.02
Lysine	0.07	0.07
Vitamins and minerals premix 1)	0.30	0.30
BHT- antioxidant 2)	0.02	0.02
Chemical composition		
A- Analyzed* (%)		
Dry matter	92.18	91.85
Crude protein	23.05	20.21
Ether extract	8.41	7.82
Crude fiber	3.37	3.38
Ash	2.36	2.23
B- Calculated **		
ME (kcal/kg)	3203.02	3210.12
Calcium (%)	1.10	0.90
Available phosphorus (%)	0.45	0.35
Sodium (%)	0.20	0.15
Met (%)	0.50	0.38
Met+ Cys (%)	0.90	0.72
Lys (%)	1.10	1.02

1) Each 3 kg of premix (TAgRO MiX®) contains: vitamin A, 12,000,000IU; vitamin D_3, 2,000,000IU; vitamin E acetate, 20,000 mg; vitaminK_3, 4,790 mg; vitamin B_1, 1,272 mg; vitamin B_2, 6,250 mg; vitamin B_6, 1,84 mg; vitamin B_{12}, 1,000 mg, biotin, 2,500 mg; pantothenic acid, 11,338 mg; nicotinic acid, 30,303mg; folic acid, 1,099 mg; manganese oxide, 432,910 mg; zinc oxide, 78,250 mg; iron sulphate,127,120 mg; copper sulphate, 20,834 mg; potassium iodide, 1,634 mg; sodium selenite, 235 mg; cobalt sulphate, 968 mg; oyster shell add to 3,000 g. 2) BHT- antioxidant: butylated hydroxytoluene used as fat antioxidant. *Based on ingredient assay before formulation. ** According to the NRC (1994) tables for composition of feedstuffs.

The birds were fed ad libitum a starter diet until 21 d of age followed by a grower diet from day 21 to day 42. Feed intake and body weight were recorded at weekly intervals from which weight gain and feed conversion of birds were calculated. Mortality was recorded daily and feed intake was adjusted for mortality. The daily ambient temperatures and relative humidity inside the experimental room were recorded at 8 am, 3pm, and 8pm. The average of temperature and relative humidity was calculated in each feeding period (Table 2).

It is difficult and expensive to build and operate climate- controlled facilities to conduct thermoneutral environments during summer months. Therefore to circumvent this problem, another group of birds which consists of 32 broiler chicks (Cobb-500) was used as a thermoneutral control group. This group was reared under natural autumn temperature (from 1 November to 12 December). The birds of this group were also divided into 4 replicates (8 birds each) and fed the same control diets. This was done to test the effect of heat stress per se on the performance and health criteria by comparing the results of this group with those of the previous heat stressed control one.

Determination of blood parameters

Eight blood samples per group (two/ replicate) were obtained by venipuncture of left wing vein at 42 day of birds' age for measurement of blood parameters. Five ml blood was taken from each bird in a sterile plastic syringe. Two ml of the blood samples were collected in sterile heparinized centrifuge tubes. They were centrifuged at 1000 g for 15 minutes for separation of blood plasma that was used for measurement of glutathione peroxidase according to the method of Mates et al. (2000). The other three ml of the blood samples were collected in clean centrifuge tubes and left at room temperature for 20 minutes to clot. They were also centrifuged at 1000 g for 15 minutes for separation of blood serum that was used for measurement of malondialdehyde “MDA”, glucose, total proteins and total cholesterol by using Biomerieux Kits (Biomerieux representative office, Egypt) according to the manufacturer’s specifications.

Determination of gross carcass traits

At the end of experiment (day 42), after an overnight fasting, 2 birds were randomly chosen from each replicate (8 birds/ group) for slaughtering to determine gross carcass traits. The feather was picked and eviscerated carcass weight (g) and yield (%) were calculated. Abdominal fat pad and liver were removed, weighed, and expressed as a percent of live body weight.

Statistical analysis

All values are presented as means ±SE. Data were statistically analyzed using Graphpad ©Instat, 1994 (software, Philadelphia, USA). The significancy of the differences between thermoneutral and heat stressed control groups was tested by Student *t*- test and among heat stressed control and supplemented groups by one way – analysis of variance (ANOVA).

Table 2. Ambient temperature (°C) and relative humidity (%) during the experimental periods in summer and autumn months

Period (days)		**Summer**		**Autumn**	
		AT	**RH**	**AT**	**RH**
0 – 21	Maximum	38.3	84.3	34.0	69.0
	Minimum	32.7	65.9	23.3	57.8
	Average	36.0	73.3	28.3	63.0
	SD	1.9	5.05	3.3	3.7
21 – 42	Maximum	39.4	88.3	26.4	76
	Minimum	31.5	68.6	20	65.3
	Average	36.5	77.6	22.8	70.9
	SD	2.5	5.4	1.93	3.2
0 – 42	Maximum	39.4	88.3	34.0	76.0
	Minimum	31.5	65.9	20.0	57.8
	Average	36.2	75.4	25.5	67.0
	SD	2.3	5.6	3.9	5.3

AT = Ambient temperature; RH = Relative humidity.

RESULTS

Growth performance parameters

Concerning the environmental temperature during the experimental periods, it was found that the ambient temperature varied from 32 to 39 ºC during the summer condition, whereas it ranged from 20 to 34 ºC in autumn (Table 2). Moreover, the relative humidity (RH) varied from 66 to 88% in summer and from 58 to 76% in autumn period.

The combination of high ambient temperature and high relative humidity severely reduced ($p < 0.001$) growth performance (Table 3). The heat stressed control group had the lower body weight gain, feed intake, and the higher and poorer feed conversion ratio as well as the higher mortality rate compared with the thermoneutral control group.

There were significant differences in body weights and weight gains between non- supplemental control group and other supplemental –groups after 3weeks of exposure to hot and humid environment (Table 3). Supplementation improved body weight and weight gain with the most favorable results (639 and 598g, respectively) recorded by vitamins group where it scored increase in gain of about 118 g representing 25% while the Cr- group exceeded only by few grams, 20g considering the least among the tested groups in comparison with control group. Body weights of broilers fed the Zn + Se supplemented diet reached 600 g at 21 days of age gained 80g more than those fed control diet. During the growing period (21-42d) supplementing diets with antioxidants (except Cr) had no significant improvement in weight gain, about 13% in vitamins group and 6% in Zn +Se group. Chromium picolinate added marked increase (p < 0.001) in weight gain by about 275g (29%) although it had no reasonable benefit during the starting phase.

Table 3. Growth performance in thermoneutral and heat stressed broilers fed diets supplemented with antioxidant nutrients (throughout the experiment; 0-42 days)

Groups / Parameters	Thermo-neutral control	Heat stress			
		Control	Vitamins”E+C”	Zn + Se	Cr
Body weight (g)[1]					
0 day	39.88±0.42	40.27±0.82	41.00±0.72	40.85±0.50	39.19±0.75
21 day	749.96±22.73[a]	520.38±10.88 [b]	639.09±9.92***	600.41±10.15**	539.49±16.49
42 day	2250.34±35.11 [a]	1466.58±30.13 [b]	1695.03±47.44**	1602.96±43.96	1760.53±25.47***
Body weight gain (g)					
0 to 21 days	710.08±23.14 [a]	480.11±13.43 [b]	598.09±10.63**	559.56±13.95*	500.30±25.67
21 to 42 days	1500.38±39.86 [a]	946.20±30.14 [b]	1055.94±29.60	1001.59±48.25	1221.04±25.92***
0 to 42 days	2210.46±35.08 [a]	1426.31±34.69 [b]	1654.03±26.96**	1561.15±36.78	1721.34±40.24***
Feed intake (g)					
0 to 21 days	920.73±34.60 [a]	859.1±29.03 [b]	909.13±39.02	889.78±37.42	824.58±38.96
21 to 42 days	2957.49±36.77 [a]	2584.4±42.3 [b]	2374.82±48.33*	2353.44±56.72**	2448.28±44.35
0 to 42 days	3878.22±39.22 [a]	3443.5±65.1 [b]	3283.95±70.92	3243.22±69.19	3272.86±45.48
Feed conversion index (feed/gain; g/g)					
0 to 21 days	1.30	1.79	1.52	1.59	1.65
21 to 42 days	1.97	2.73	2.25	2.35	2.01
0 to 42 days	1.75	2.41	1.99	2.08	1.90
Mortality (%)					
0 to 21 days	3.13	12.50	0.00	6.25	3.13
21 to 42 days	3.23	21.43	9.38	6.67	9.68
0 to 42 days	6.2 5	31.25	9.38	12.50	12.50

[a,b] The comparison between thermoneutral control and heat stressed control groups revealed highly significant differences (p < 0.001). *, **, *** indicates significant at p < 0.05, p< 0.01 & p < 0.001, respectively, when the supplemented groups compared with heat stressed control group. [1] body weight was measured weekly, but the results of days 0, 21 and 42 only are presented in the table.

All over the 42days- period, weight gain was significantly increased with the addition of Cr (p < 0.001) and vitamins C+E (p < 0.01) while showed insignificant improvement with Zn + Se supplement.

Feed intake was not significantly influenced by dietary supplementation during the first 3 weeks of rearing period where either the increase in amounts of food consumed by vitamins and Zn + Se groups did not reach 10% (3.6 to 7.2) or the decrease was about 4% in Cr- group. In the next 3 weeks, growing period, birds receiving supplements independent of type had lower feed intake by about 210 & 231g in vitamins and Zn+ Se groups, respectively. These reductions overlapped the increase in feed intake in the starting period so it gave net reduction 160g in vitamins and 200g in Zn + Se group in the entire experimental period (0 - 42d). Birds in Cr- group consumed less feed all over the experimental period.

Concerning the feed conversion rate, during the first three weeks of feeding period the supplementation of antioxidant nutrients improved the feed utilization by the rate ranged from 1.52 in vitamin group to 1.65 in Cr group while the Zn+Se group occupied the intermediate position (1.95) compared with thermoneutral control group (1.79). In the period from 21 to 42 days, although the feed intake of broilers fed supplemented diets decreased, the birds efficiently utilized the food in term of FCR more than the control group (2.25, 2.35 &2.01 versus 2.73). When the whole experimental period was taken into account, supplementing the diet with vitamins C+E and organic Cr significantly improved the feed conversion of birds at the ratio of 1.99 and 1.90, respectively. Birds in Zn+ Se group converted the feeds to gain by efficiency 12% more than the control group (2.41).

As a general, mortality rate (Table 3) increased under heat stress condition and the last week of the experimental period showed the highest rate. In the control group, the mortality rate represented about 12.5% in the first 3 weeks of age and then increased reaching 21.4% during the period of 21 to 42 days and totaled nearly 31% in the whole raising period. While under thermoneutral condition only two birds died representing about 6% during the whole experimental period. In this study the supplementation of antioxidants improved the viability and decreased the mortality to the degree that no deaths occurred in the vitamin group and in the other groups the rate was less than that in the heat stressed control where it was 3% in Cr- group and 6% in Zn+ Se group during the first three weeks of treatments. Although the deaths increased during the period of 21 to 42 days, the rates were less than in the heat stressed control group. In the total period of 42 days, the mortality rate was 9.4, 12.5 & 12.5% in vitamin-, Zn + Se -, and Cr- supplemented groups, respectively.

Blood parameters

The comparison of the blood metabolites and oxidative stress parameters in thermoneutral control and heat stressed control groups (Table 4) demonstrated that the heat stress significantly ($p < 0.001$) increased cholesterol, glucose, and malondialdehyde and decreased protein and glutathione peroxidase. Moreover, the supplemented treatments improved the blood profile parameters and the oxidative status of the birds as it was obviously seen by a significant decrease in cholesterol, glucose, and malondialdehyde and increase in protein and glutathione peroxidase especially in Cr-supplemented group.

Measurement of Malondialdehyde as an oxidative stress indicator in the serum of the tested bird groups revealed a significant increase ($p < 0.001$) in its concentration in the heat stressed control group 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 (Table 4).

Assessment of GPx activity in the blood plasma of the birds as one of the antioxidant enzymes showed that there was a significant decrease in its activity in the heat stressed control group. However, the supplemented diets improved these negative impacts of the heat stress by a significant increase ($p < 0.05$) in GPx activity in all the supplemented dietary groups (Table 4).

Table 4 also showed that the heat stress significantly increased ($p < 0.001$) the serum total cholesterol and blood glucose levels, but decreased ($p < 0.001$) the total serum protein level. This is indicated when the heat stressed control group was compared with the thermoneutral control one. Although dietary supplementation with vitamins “E+C” microminerals, “Zn+Se” or Cr succeeded in reduction of the adverse effects of heat stress on most of the tested blood parameters, these supplements had insignificant beneficial effect on cholesterol level (in Zn + Se group) and on total serum protein (in vitamins group).

Carcass traits

The carcass and liver yields decreased by the effect of heat stress as shown in the heat stressed control group and the dietary supplementation did not add marked improvement impact (Table 5). The great effect of the heat stress on the carcass quality appeared in the increase of visible abdominal fat in the heat stressed control group to about 2.78% which decreased to 2.30% with each of the additional vitamins and organic Zn + Se and to 2.20% with Cr piclionate.

Table 4. Blood parameters in thermoneutral and heat stressed broilers fed diets supplemented with antioxidant nutrients (at the end of the experiment; d 42)

Groups / Parameters	Thermo-neutral control	Heat stress			
		Control	**Vitamins”E+C”**	**Zn + Se**	**Cr**
Plasma:					
GPx (U/L)	2.85±0.08 [a]	2.13±0.07 [b]	2.71±0.09**	2.69±0.11**	2.47±0.09*
Serum:					
MDA (µmol/ml)	1.93±0.05 [a]	3.10±0.09 [b]	2.33±0.08***	2.28±0.07***	2.50±0.08***
Protein (g/dl)	3.70±0.08 [a]	2.89±0.06 [b]	3.05±0.08	3.43±0.06***	3.30±0.05**
Cholesterol (mg/dl)	127.47±5.0 [a]	176.47±7.29 [b]	145.19±5.96*	152.92±6.85	129.50±6.40***
Glucose (mg/dl)	214.58±7.21 [a]	280.24±6.18 [b]	244.46±5.29*	237.43±7.41**	225.20±7.55***

[a, b] the comparison between thermoneutral control and heat stressed control groups revealed highly significant differences ($p < 0.001$). *, **, *** Indicates significant at $p < 0.05$, $p< 0.01$ & $p < 0.001$, respectively, when the supplemented groups compared with heat stressed control group.

Table 5. Carcass characteristics in thermoneutral and heat stressed broilers fed diets supplemented with antioxidant nutrients (at the end of the experiment; d 42)

Groups / Parameters	Thermo-neutral control	Heat stress			
		Control	**Vitamins”E+C”**	**Zn + Se**	**Cr**
Carcass yield (%)	72.32	69.07	70.25	71.78	71.54
Liver yield (%)	2.23	2.57	2.40	2.45	2.30
Abdominal fat yield (%)	1.80	2.78	2.32	2.34	2.15

DISCUSSION

Growth performance

Birds kept in heat stress condition during summer months (36˚C and 75% RH) presented about 35% lower weight as compared to those in thermoneutral condition (25˚C and 67% RH) which is explained by a 11.2% reduction in feed intake ($p < 0.01$). This result was in accordance with the general trend observed in heat stressed broilers (Austic, 1985 and Geraert et al., 1996). It is believed that for every 10˚C increase in ambient temperature above 20˚C, there is a 17% reduction in feed intake (Austic, 1985) and above 22˚C the reduction was 24% (Koh and Macleod, 1999). Geraert et al. (1996) observed a 14% reduction in body weight from 2 to 4 weeks of age and a 24% reduction from 4 to 6 weeks of age when birds were exposed to 32˚C.

As observed, the proportion of reduction in body weight gain was greater than the proportion of reduction in feed intake (-36 versus -11%) for heat-exposed broilers, leading to poor feed conversion. These results indicated that the decreased body weight is not only due to the lower feed intake, but also to a direct effect of environmental temperature on broiler physiology and metabolism as cited by Ain Baziz et al. (1996) and Geraert et al. (1996).

Growth performance was also reduced due to the harmful effect of lipid peroxidation as a consequence of increased free radical generation and this was demonstrated by a significant increase in serum MDA level and lower activity of GPx in heat stressed control group.

Previous studies on vitamins C and/or E have shown that the growth was not significantly influenced by vitamin E (Niu et al., 2009) or by vitamin C (Konca et al., 2009) or by a combination of them (Lagana et al., 2007) which doesn't agree with the present data. Birds in trace elements (Zn+Se) - group achieved non-significant increase in body weight (135g). Such finding coincides with that obtained by Upton et al. (2009) and Khajali et al. (2010) but is in contrast with Combs and Combs (1986) and Choct et al. (2004).

Totally, in the 42d- experimental period birds significantly gained weights of about 300g with the addition of chromium and 230g with vitamins. Similar positive effect of Cr supplementation on body weight and gain was recorded by Naghieh et al.(2010) with 600µg/kg Cr nicotinate, Noori et al. (2012) with Cr methionine from 200 to 800 and by Toghyani et al.(2012) with 1500ppb organic or inorganic Cr.

The dietary supplements reduced feed intake all over the experimental period. These findings are in a close agreement with that presented by Konca et al. (2009) who observed that the supplementation of vitamin C to the broiler diets significantly decreased daily feed intake at 21-42 and 0-42 days of age. Also the reduction in feed intake was recorded by Lagana et al. (2007) with the addition of vitamins C& E or organic Zn & Se. On the contrary, appetite increased and birds consumed more diets supplemented with vitamin C (Kutlu and Forbes, 2000) or with Cr (Sahin et al., 2002; Toghyani et al., 2006; Naghieh et al., 2010). There was no significant effect recorded with the supplementation of vitamin E (Niu et al., 2009); Se (Upton et al., 2009 and Khajali et al., 2010) or Cr (Anandhi et al., 2006 and Jackson et al., 2008).

As observed from the results in Table 3 that the improvement in the body weight gains was greater than or did not meet by improvement in feed intake and this may be caused by the improved effect of the dietary supplementation on feed utilization, broiler physiology and metabolism as cited by Ain Baziz et al. (1996) and Geraert et al. (1996). Efficiency of broilers in feed conversion was affected by feed additives where the addition of vitamins C+E and microelements Zn +Se decreased the cost of kg gain by consuming 0.27& 0.20 kg less food.

The positive effect of Se and Zn supplement on feed conversion may be attributed to the improvement in nutrient digestibility and efficiency of its use (Lagana et al., 2007 and Sahin et al., 2009) and therefore, decreased feed intake and improved feed conversion ratio. Because Zn and Se have a protective role on pancreatic tissue against oxidative damage, it may help the pancreas to function properly including secretions of digestive enzymes, thus improving digestibility of nutrients and consequently, performance. In the same trend diets supplemented with Cr (Sahin et al., 2003; Jackson et al., 2008; Adebiyi and Makanjuola, 2011), or vitamin E (Guo et al., 2003;Lohakare et al., 2005; Niu et al., 2009) were efficiently converted into weight gain. However, previous studies to test the effect of the supplementation of Zn (Bartlett and Smith, 2003), Se (Upton et al., 2009 and Khajali et al., 2010), Cr (Toghyani et al., 2006, Naghieh et al., 2010, Noori et al., 2012), vitamin E (Niu et al., 2009) and vitamin C (Marron et al., 2001; Konca et al., 2009) revealed no significant effects under high temperatures.

The mortality rates were severely affected by the heat stress condition, especially during the last week of the experiment. This agrees with the previous studies which showed that bird mortality increased during heat stress (Arjona et al., 1990, De Basilio et al., 2001and Roussan et al., 2008) and was greater near the marketing time where heavier weights (Arjona et al., 1990 and Roussan et al., 2008). Supplementation of antioxidants decreased the mortality rate throughout the experiment. This was clearer in the vitamins supplemented group which exhibited the lowest mortality rate comparing to the other groups. Roussan et al. (2008) reported that there was significantly lower mortality rate under cycling heat stress when vitamin C was supplemented.

Blood parameters

Heat stress increased lipid peroxidation as a consequence of increased free radical generation. The rise of lipid peroxidation resulted in increased MDA level in blood and tissues (Okutan et al., 2005 and Ates et al., 2006). This also was recorded in the heat stressed control group of this study where serum MDA level was 3.1µmol /ml which differed significantly from thermoneutral control group (1.93µmol/ml). The level significantly reduced ($p < 0.001$) by dietary treatments to about 2.3 µmol/ml in each of vitamin C+ E and Zn+ Se and 2.5 µmol/ml in Cr groups (Table 4). Consistent with our results, Tatli Seven et al. (2009) found that plasma MDA level was significantly decreased in Vit. C- group compared to the control group (13.4 vs. 16.3nmol/ml). Separately or as a combination, supplemental vitamin C and chromium resulted in a decrease in MDA concentration (Sahin et al., 2003). Similar effect was achieved with dietary supplementation of vitamin E (Morrissey et al., 1997 and Sahin et al., 2001). Vitamin E is the first line of defense against lipid peroxidation. By its free radical quenching activity, it breaks chain propagation and thus terminates free radical attack at an early stage (McDowell, 1989). Also Zn supplementation decreased serum and liver MDA levels in heat stressed birds and this might be attributed to that Zn induces production of metallothionein, which is an effective scavenger for hydroxyl radical (Sahin et al., 2009). Another mode of action proposed for Zn as an antioxidant is its interaction with vitamin E because vitamin E status is impaired in Zn- deficient animals and supplementation of vitamin E prevented some of oxidative damage lesions (Kim et al., 1998).

GPx, present in the cytosol and mitochondrial matrix, catalyzes the degradation of various peroxides by oxidizing glutathione. Se is an essential component of Se-dependent glutathione peroxidase enzyme, which reduces peroxide and protects cells against the damaging effects of oxidation (Reddy et al., 2009). Jianhua et al. (2000) and Payne and Southern (2005) recorded that dietary Se supplementation increased the plasma GPx activity in the broiler chickens. Khajali et al. (2010) found that the inclusion of organic Se source (selenomethionine) significantly elevated plasma GPx activity when measured at 40 days of bird's age, which can be regarded as an improvement of antioxidant status. In addition to the role of Se as antioxidant, methionine moiety can be converted to cysteine which in turn, converts to GSH. Both cysteine and GSH can function as direct scavengers of reactive oxygen species (ROS). GSH and cysteine can also protects proteins from irreversible oxidative damage through interactions between these thiols and proteins and the formation of mixed disulfides, such as glutathiolated protein (Mallis et al., 2002). Vitamin C also significantly increased levels of GPx in blood, and tissues (Tatli Seven et al., 2009).

The blood components are particularly sensitive to changes in ambient temperature, being an important indicator of physiological responses in birds to stressing agents. During heat stress, there was greater catabolic effect and concentration of adrenocorticotropic hormone yielding more glucose, uric acid, and triglycerides in serum.

The increase in glucose concentration is directly responsive to an increase in glucocorticoids (Borges et al., 2007), which can result from various stressors including heat stress. Glucocorticoids have primary effects on metabolism, stimulating gluconeogenesis from muscle tissue proteins. Kutlu and Forbes (1993) and Rashidi et al. (2010) reported that high environmental temperature increased levels of plasma glucose and cholesterol and reduced protein level. Comparable results were obtained in this study where concentration of serum glucose and cholesterol, mg/dl increased by 31 & 38 percent while the protein level decreased to 78% in heat stressed control. The increase in blood lipids under heat stress was explained by Rashidi et al. (2010) that high temperature reduced feed intake and broilers compensate their need to energy by lipolysis of body lipid that it causes increasing the blood cholesterol and triglycerides. On the other hand, Tatli Seven et al. (2009) recorded that glucose, total protein (Tatli Seven et al., 2009; Rashidi et al., 2010), total cholesterol, VLDL cholesterol, and triglycerides in blood plasma were not significantly influenced by heat stress.

Previous attempts to reduce the detrimental effects of heat stress indicated that Zn supplement had a significant effect on cholesterol concentration of plasma ($p < 0.05$) and its level linearly decreased as dietary Zn supplementation increased from 30 to 60mg/kg (Sahin et al., 2005). Kucuk et al. (2003) reported that Zn resulted in an increased total serum protein but decreased glucose and cholesterol concentrations. Similar results were obtained with the supplementation of vitamin C by Kutlu and Forbes (1993), Sahin et al. (2003) and Gursu et al. (2004) and with Cr by Sahin et al. (2002) in broilers and quails. On the contrary to these findings the blood parameters were not influenced by the addition of vitamin C (Konca et al., 2009 and Tatli Seven et al., 2009) or Cr (Adebiyi et al., 2011and Toghyani et al., 2012).

These earlier researches can introduce a proof to the present results (Table 4) which showed that supplementation of Zn + Se and Cr significantly affected serum total protein by increasing its level 1.19 and 1.14 times the value in the heat stressed control group (2.89g/dl), respectively. Dietary vitamins C + E caused insignificant improvement.

Serum cholesterol level significantly decreased from 176.5mg/dl in heat stressed control group by about 27and 18% with the supplementation of Cr and vitamins, respectively. In addition, there was insignificant reduction in cholesterol level of Zn + Se - supplemented group.

Serum glucose level was significantly lowered in Cr; vitamins; and Zn + Se groups. These values were ranged from 80 to 87% of that in the control group (280 mg/dl). 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.

Carcass traits

Carcass and liver yields were adversely affected by high temperature where they represented 96% of that of normal control group. Dietary supplementation had no markedly improved effect on carcass or liver yield and are in agreement with some studies which suggested that carcass traits were hardly influenced by dietary modulations; carcass and heart yields were not affected by supplementation of vitamin C (Konca et al., 2009; Celik and Ozturkcan, 2003) and Se (Khajali et al., 2010) and liver and gizzard yields also were not affected by vitamin C (Konca et al., 2009). Also no effect was exhibited by Cr supplementation on carcass trait (Jackson et al., 2008). However, others reported that dietary vitamin C (Sahin et al., 2002; Sahin et al., 2003; Lohakare et al., 2005), Cr (Sahin et al., 2002), and zinc (Kucuk et al., 2003) supplementation significantly increased carcass weight and yield as well as the weights of internal organs.

The increase in carcass fat of broilers raised under hot ambient temperature is another concern since the fat content of meat products has become increasingly important to consumer perceptions of the healthfulness of meat. The current results in Table 5 showed that abdominal fat pad yield increased from 1.8 in normal control to 2.78% by more than 50% in heat stressed control group. This supports the results of Ain Baziz et al. (1996) and Mendes et al. (1997).

The dietary supplements had significant effect where fat yield decreased to an average 2.3 % in each of vitamins -, and Zn & Se - group and to 2.2 in Cr-group in comparison with heat stressed control group. Previous studies had similar results that abdominal fat pad decreased upon the addition of vitamin C (Sahin et al., 2002; Sahin et al., 2003), Cr (Gursoy, 2000; Sahin et al., 2002; Toghyani et al., 2012), Zn (Kucuk et al., 2003), and Se (Konca et al., 2009). Vadhanavikit and Ganther (1994) indicated that Se supplementation declined the activity of cytosolic malic enzyme leading to decline in abdominal fat deposition. On other hand no obvious effect of supplemental Se (Khajali et al.,

2010) and vitamin C (Konca et al., 2009; Celik and Ozturkcan, 2003) was found.

CONCLUSION

As known, heat stress affects adversely the productive performance in broilers. The control of high environmental temperature is difficult due to the high cost and impractical of cooling bird buildings. Therefore, dietary manipulations are considered to be the beneficial and economical methods to alleviate the negative effects of heat stress. The present results found that supplementation of the diets with antioxidants, especially vitamins and chromium, is essential to overcome the deleterious effects of heat stress conditions on the oxidative status and performance of broilers.

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