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Recovery from Adverse Effects of Heat Stress on Slow-Growing Chicks Using Natural Antioxidants Without or with Sulphate

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Abstract: An experiment was designed to study the effect of *Curcuma longa* (CL), *Cuminum cyminum* L(CC) alone or with sulphate ion on alleviating the heat stress effects compared to vitamin C. Three hundred and sixty, 21-day old unsexed chicks of slow growing El-Salam strain were randomly divided among 8 treatments (each of 3 replicates of 15 unsexed chicks each) and housed in floor pens. One group was kept under thermoneutral condition at 28±4°C and 55±3% Relative Humidity (RH) during 21-84 day of age and fed practical corn-soybean meal diet (control diet). The other seven groups were kept for three successive days weekly under heat stress at 38±1.4°C and 49±2% RH from 12.00 to 16.00 pm. Chicks in Heat Stress treatments (HS) were fed basal diet without additives or with 250 mg Ascorbic Acid (AA) /kg diet, 0.2% of *Curcuma longa* (CL),0.2% *Cuminum cyminum* L (CC), 0.5% anhydrous Sodium Sulphate (SS), 0.2% CL+0.5%SS and 0.2%CC+0.5%SS. Heat Stress decreased body weight gain, feed intake, feed conversion, carcass percentage, nitrogen retention, ash retention and plasma antioxidants capacity while increased respiration rate (RR) and rectal temperature (RT). The addition of CL, CC alone or with SS in the diet can recover the negative effect of HS on performance, nitrogen retention, ash retention and plasma antioxidants capacity. To some extent these additives recover the negative effect on RR and RT. The *Cuminum cyminum* L plus sulphate seemed to be the best additive under the condition of this study.

Key words: *Curcuma longa*, *Cuminum cyminum*, sulphate ion

INTRODUCTION

High ambient temperature is a problem in many parts of the world. Heat stress has been associated with decreases in broiler weight gain, feed intake, feed efficiency, nitrogen retention, protein digestibility and total mineral retention (Austic, 1985; Sahin and Kucuk, 2003).

Acute heat stress caused a reduction in initial breast muscle pH in broiler chickens (Sandercock *et al*., 2001) and resulted in accelerated rigor mortis development, reduced water-holding capacity and increased paleness of breast meat (Northcutt *et al*., 1994; McKee and Sams, 1997). Environmental stress causes oxidative stress and impairs antioxidant status *in vivo* (Halliwell and Gutteridge, 1989; Sahin *et al*., 2001). Mujahid *et al*. (2005) shown that superoxide production by the skeletal muscle mitochondria of meat type chickens is significantly enhanced by heat stress. This in turn was associated with a heat-induced increase in rectal and muscle temperatures, leading to a significant body weight loss. Antioxidant vitamins and minerals such as vitamin C, E, A and Zn have been used to ameliorate the effects of environmental stress (Sahin and Kucuk, 2003). Turmeric (*Curcuma longa*, CL), a medicinal plant native to the Asian subcontinent, is known to possess antimicrobial and antioxidant properties. *Curcuma* spp contain turmeric (a water-soluble peptide), essential oils (such as zingiberene) and curcminoids including curcumin (Sharma *et al*., 2005). The curcuminoids, yellowish pigments present in turmeric powder, have shown protective effects against aflatoxin B1 (Soni *et al*., 1997). Further, curcumin has a strong inhibitory action on superoxide anion generation (Iqbal *et al*., 2003). *Cuminum cyminum* L (CC) is an annual plant of the Umbelliferae family. This plant, which is one of the important spices in the world, is native to Egypt. CC is used as a condiment and as an ingredient in many food industries. Cumin aldehyde was found as the main component in cumin seed oil from Egypt (Shaath and Azzo, 1993). Birjees Bukhari *et al*. (2009) suggested CC to be a potent source of antioxidants. It is known that the measurement of high antioxidant capacity in foods may or may not be an indication of the potential for altering *in vivo* antioxidant status. The bioactive phytochemicals in foods have varying bioavailability and may influence biological processes thus, it is important to understand whether sufficient quantities of antioxidant phytochemicals can be absorbed in a form that might alter *in vivo* antioxidant status. Falany (1991) showed that sulfation has evolved as a key step in xenobiotic metabolism. Azuma *et al*. (2000) found that absorbed flavonoids are present in the common blood circulation in the form of glucuronide, sulphate and methylate conjugates are excreted via urine or bile while

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chlorogenic acid (important phenolic antioxidants) remained intact in the small intestine. It is known that sulphate play role in detoxification and metabolism the phenolic compound (Ali, 2005). Yeh and Yen (2006) showed that the supplementation of natural phenolic acids through a balanced diet containing enough fruits and vegetables could be the most effective in inducing of phase II sulphate conjugation enzyme. Ninfali *et al.* (2005) suggest that not all of the ingested phenolics can reach the plasma, but those escape in blood stream lead to a significant increase in total plasmatic antioxidant capacity, both when they are in the free a gluconic form and when they are glucuronide or sulphate conjugated form. Bartelt and Kirinya (1976) found that liver slices from geese, ducks and chickens contained high sulphate conjugation enzyme activities. The addition of sulphate to herbs may increase the activity of its antioxidants properties. Ali *et al.* (2007) showed that combination of thyme and sulphate is the most successful additive for improving hatchability and indicated that heavier strains need sulphate to increase the response to thyme. The present study was designed to study the effect of CL or CC alone or with sulphate ion on alleviating the heat stress using slow growing chicks compared to vitamin C.

MATERIALS AND METHODS

Three hundred and sixty, 21-day old of slow growing unsexed chicks white feathers crossbred of El-Salam local strain were weighed and equally distributed among eight groups, each group contained three replicates; each replicate (1 x 1.5 m^2) consisted of 15 birds. During the experimental period (21-84 day of age), the positive control group was kept under thermo neutral condition in semi-opened house and fed control diet (Table 1), the average temperature was 28±4°C and RH was 55±3%. While, the other seven groups were kept for three successive days per week under 38±1.4°C and 49±2% RH from 12.00 to 16.00 pm. The heat source was provided by gas heaters supplemented with thermometer. Chicks in heat stress treatments were fed control diet (HS) without additives or with 250 mg Ascorbic Acid/kg diet (AA), 0.2% of *Curcuma longa* (CL), 0.2% *Cuminum cyminum* L(CC), 0.5% anhydrous Sodium Sulphate (SS), 0.2% CL+0.5%SS and 0.2%CC+0.5%SS. The birds were individually weighed (BW) and calculated Weight Gain (WG) every 3 weeks. Simultaneous Feed Intake (FI) and Feed Conversion (FC) on replicate basis were also recorded. Anhydrous Sodium Sulphate (SS) was supplied by the Egyptian Salt and Mineral Company. The CL and CC were purchased from local market in Cairo. At the end of the experiment (12 week of age), 6 birds of each treatment, as three of each sex, were slaughtered to determine carcass characteristics. Chemical analyses for protein, lipids, and ash were done in skinless-boneless pooled samples (50:50; weight/weight) of breast plus thigh muscle according to AOAC (1995). Meat quality measurements such as meat tenderness and Water Holding Capacity (WHC), meat colour intensity and pH value were determined as outlined by Attia (2003). At the end of the experimental period three birds from each group were housed in separate metabolic cages for 5 days. After a 3 days preliminary period, feed intake and excreta were measured and collected during 5 days. The proximate analyses of feed and dried excreta were determined according to AOAC (1995). Individual blood samples were taken from 3 birds within each treatment, and collected into dry clean centrifuge tubes containing drops of heparin and centrifuged for 20 min (3000 rpm) for obtaining plasma. Antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Plasma total lipids, total protein, albumin, cholesterol, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), calcium, phosphorus and zinc were determined by suitable commercial kits. The globulin values were obtained by subtracting the values of albumin from the corresponding values of total protein. Blood pH, Hemoglobin (Hgb) and Packed Cell Volume (PCV) were determined during the 5th week on eight birds/treatment just before end of HS. Heparinized blood samples were

Table 1: The composition and chemical analysis of control diet.

Ingredients	%
Yellow corn	63.05
Soybean meal (44%)	33
Dicalcium phosphate	2
NaCl	0.3
Limestone	0.9
Vit. + Min. Mix.*	0.3
Vegetable oil	0.3
Methionine	0.1
Lysine	0.05
Total	100
Calculated values**	
ME (Kcal/Kg)	2900.8
Ca (%)	0.9
Av. Phos. (%)	0.45
Methionine (%)	0.42
Methionine + Cystine (%)	0.75
Lysine (%)	1.1
Determined analysis	
CP (%)	19.3
DM (%)	89.71
EE (%)	4.66
CF (%)	3.05
Ash (%)	9.73

*3 kg of vitamin-mineral premix per ton of feed supplied each kg of diet with Vit. A 12000 IU; Vit. D3 2000 IU; Vit. E. 10 mg; Vit. k3 2 mg; Vit. B1 1 mg; Vit. B2 4 mg; Vit. B6 1.5 mg; Pantothenic acid 10 mg; Vit. B12 0.01 mg; Folic acid 1 mg; Niacin 30 mg; Biotin 0.05 mg; Choline chloride (50% choline) 500 mg; Zn 55 mg; Fe 30 mg; I 1 mg; Se 0.1 mg; Mn 55 mg; ethoxyqain 3000 mg. **calculated values were according to NRC (1994).

taken (~3 ml) from the brachial vein to determine pH value using digital electric pH meter immediately after collection of samples. Hemoglobin concentration (Hgb) was detected as g/dL by the cyanomethemoglobin procedure (Eilers, 1967). Heparinized blood was used for determination of PCV using Wintrobe hematocrit tubes. Blood sample were centrifuged for 20 min at 4,000 rpm then PCV values were obtained by reading the packed cell volume on the graduated hematocrit tubes. Sheep Red Blood Cells (SRBCs) were used as an antigen test to quantitatively analyze the humoral immune competence. Eight chicks per group were immunized i.v. via a wing vein with 1 ml SRBCs solution of 10% SRBCs suspension in sterile saline. The chicks were injected just before heat regime of the first day. At 3, 6 and 9 days post-immunization, ~2.0 ml blood samples were collected. The levels of antibody were determined using a micro hemeagglutination technique as cited by (Kai *et al*., 1988). At 2,5 and 8 Week the respiration rate was measured by counting the breath/minute through observing the abdominal movement for one minute. At 2, 5 and 8 week rectal temperature was monitored by thermo code electric gauge with accuracy of 0.1°C. Data were analyzed by GLM of SAS® (SAS Institute, 1990; Cary, NC, USA) using one-way model. Duncan's multiple range test (Duncan, 1955) was used to test the significance ($p<0.05$) of differences among means.

RESULTS AND DISCUSSION

Productive performance: Data in Table 2 shows the effect of different dietary treatments on productive performance of chicks. There were significant ($p<0.01$) differences between values of BW, WG, FI and FC recorded by different treatments. HS decreased BW, WG and FI and impairs FC and these results agree with those previously reported by (Geraert *et al*., 1996; Temim *et al*., 1998). The BW of HS group was lower about 16.19% than those of control group and these results agree with those reported by Bohren *et al*. (1981) who found that chronic heat stress has been shown to result in a 20% reduction in growth rate in fast-growing strains of poultry while in this study we used slow growing chicks. In this respect, Cahaner and Leenstra (1992) showed that the negative effect on growth rate was found to be greater in broilers with a higher genetic potential for growth rate than in broilers with lower growth rates. Addition of CL and CC either alone or with sulphate caused an increase in WG. The beneficial effect of AA, CL or CC supplementation can be explained on the basis that heat stress caused increase of free radicals production (Halliwell and Gutteridge, 1989) and lowers the concentrations of antioxidant vitamins such as vitamins A, C and E, as well as minerals such as zinc and chromium in serum (Feenster, 1985; Sahin and Kucuk, 2003) and the additives used in this study are known to be potential antioxidants that scavenges the free radicals generated by heat stress. Adding of AA and different additives increased FI and improved FC compared to HS group. Using CL or CC in this study tend to increase the FI numerically compared to HS birds and these results may be due to stimulating enzyme activity by these additives and consequently increased FI. Muthamma Milan *et al*. (2008) found that hot water and saline extracts of CC show significant increase in amylase, proteolytic, lipase and phytase activities. Platel and Srinivasan (1991) showed that the digestive stimulatory action of spices may be through stimulation of activities of enzymes that participate in digestion. The superior in WG for group fed CC compared to CL group may be due to different in their antioxidants activity *in vivo*. Birjees Bukhari *et al*. (2009) suggested that the CC to be a potent source of antioxidants. The addition of SS alone succeeded in decreasing the bad effect of heat stress on performance of chicks. These results agree with those obtained by Ahmad *et al*. (2005) who found that Sodium sulphate increased body weight gain, feed intake and improved feed to gain ratio than those of potassium carbonate and potassium sulphate after 42 days of age under cyclic heat stress. In this study SS supplementation to CC numerically increased chick bodyweight gain and improved feed conversion while it had no response with CL and this may be due to difference molecular structure of the two additives. However, Kroon *et al*. (2004) showed that polyphenols are present in plasma and

Table 2: The effect of dietary treatments on productive performance.

ITM	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
WG3-6	353a±1.77	325c±2.03	345b±1.69	345b±1.67	348b±1.55	348b±1.30	348b±1.29	347b±1.25
WG6-9	354a±2.83	245e±2.39	325b±2.42	285d±1.86	324b±1.93	308c±1.92	314c±1.89	322b±1.58
WG9-12	342b±3.39	308d±2.62	354ab±5.95	358a±6.03	340b±4.48	326c±2.70	323c±3.48	350ab±6.19
WG3-12	1050a±2.62	880d±2.10	1024b±5.63	989c±5.74	1013b±4.09	983c±2.19	986c±2.87	1020b±5.78
FI 3-6	1161a±5.92	1108d±4.40	1161a±6.00	1140bc±5.77	1160a±5.77	1140bc±5.77	1133c±4.40	1153ab±6.00
FI 6-9	1291a±6.00	1246ab±28.3	1190b±2.0	1218b±28.3	1190b±1.00	1218b±28.00	1218b±3.00	1190b±1.00
FI 9-12	1350a±11.45	1320bc±5.77	1350a±11.54	1341ab±7.26	1350a±5.77	1300c±5.77	1305c±8.66	1310c±5.77
FI 3-12	3803a±19.29	3675b±22.54	3701b±11.66	3700b±30.41	3700b±2.00	3658b±23.51	3656b±36.6	3653b±9.27
FC3-6	3.28ab±0.09	3.40d±0.02	3.37cd±0.01	3.30abc±0.02	3.33bc±0.03	3.26ab±0.02	3.25a±0.01	3.32abc±0.01
FC6-9	3.65a±0.07	5.07d±0.09	3.65a±0.009	4.27c±0.13	3.66a±0.03	3.95b±0.10	3.88ab±0.09	3.68a±0.02
FC9-12	3.94abc±0.04	4.28d±0.02	3.82ab±0.09	3.75a±0.10	3.96bc±0.04	3.99bc±0.03	4.03c±0.02	3.74a±0.07
FC3-12	3.62ab±0.02	4.18d±0.03	3.61a±0.02	3.74c±0.02	3.65abc±.01	3.72c±0.03	3.71bc±0.03	3.58a±0.02

a-d Means in the same row with different letters, differ significantly ($p<0.05$).

tissues as conjugation with glucuronate or sulphate. It was surprise that addition of SS to CC improved FC in all over period by 1.10% compared to control diet. Mujahid *et al.* (2005) showed that superoxide production by the skeletal muscle mitochondria of meat type chickens is significantly enhanced by heat stress. Bottje *et al.* (2004) found that in breast muscle, liver and duodenal mitochondria isolated from low feed efficiency broilers exhibited higher rates of H_2O_2 production and higher protein oxidation compared to mitochondria from their age-matched high feed efficiency broilers. The addition of CC+ SS to the diet may scavenge the free radicals generated by heat stress leads to improve FC. Sinurat *et al.* (1987) reported that the efficiency of feed utilization and feed intake in broilers was reduced under heat stress and supplementation of antioxidants improved the feed efficiency of broilers. Based on the previous reasons, CC+ SS under condition of this study was suitable additive.

Carcass characteristics: The data in Table 3 indicated that there were significant ($p<0.01$) differences between dietary treatments in carcass percentage. Heat stress decreased the carcass percentage of HS and SS birds as compared to other treatments. The negative effect of heat stress on carcass has been documented by Sahin and Kucuk (2001). As shown in Table 3 the addition of AA , CL or CC plus SS diminish the negative effects of heat stress on carcass percentage. The beneficial effect of AA on carcass percentage have been reported by Sahin and Kucuk (2001) who found that vitamin C supplemental increased performance and yields better carcass traits in broilers reared under conditions of heat stress (32°C). There were significant ($p<0.01$) differences between different treatments in liver, pancreas and proventriculus percentage. Also significant ($p<0.01$) differences of bursa gland weight percentage recorded by different treatments. HS group recorded the lowest percentage and this means suppression of the immune system. Bollengier-Lee *et al.* (1998) showed that heat stress can negatively affect the defense mechanism in poultry, which can lead to suppression of the immune system . From this study, it is clear that adding of AA, CC, CL+SS and CC+SS act to increase significantly the weight percentage of bursa.

Chemical composition and quality of meat: The data in Table 4 indicated that there were insignificant differences between experimental treatments in chemical composition and quality of meat. These results disagree with those obtained by Attia *et al.* (2009) who found that HS decreased meat WHC, whilst AA restored it to that of the control group. However, Young *et al.* (2003) reported that increased antioxidative status obtained through supplementation did not improve WHC of chicken meat but increased the oxidative stability of lipids.

Nitrogen and ash retention: There were significant ($p\leq0.05$) differences between nitrogen retention values recorded by different treatments (Table 5). The HS group recorded significantly lower nitrogen retention

Table 3: The effect of dietary treatments on carcass characteristics.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
Carcass %	70.16a±0.19	67.50e±0.03	69.54b±0.03	67.96d±0.02	69.04c±0.04	67.70e±0.03	68.99c±0.04	69.57b±0.04
Liver %	2.43ab±0.01	2.10c±0.08	2.37b±0.01	2.34b±0.02	2.54a±0.03	2.32b±0.01	2.55a±0.02	2.49a±0.01
Heart %	0.44±0.01	0.42±0.009	0.47±0.009	0.44±0.004	0.44±0.01	0.44±0.004	0.45±0.005	0.44±0.007
Gizzard %	2.42±0.03	2.40±0.008	2.47±0.04	2.41±0.05	2.44±0.006	2.43±0.003	2.48±0.02	2.43±0.008
Pancreas %	0.18ab±0.007	0.16c±0.002	0.18bc±0.002	0.18ab±0.002	0.19a±0.002	0.16c±0.003	0.17bc±0.002	0.17bc±0.004
bursa %	0.26a±0.004	0.22c±0.002	0.24ab±0.004	0.23bc±0.009	0.25ab±0.004	0.23bc±0.008	0.24ab±0.006	0.24ab+0.005
Thymus	0.23±0.003	0.22±0.002	0.23±0.003	0.23±0.004	0.23±0.008	0.23±0.004	0.22±0.005	0.23±0.005
Proventriculus %	0.45a±0.005	0.42c±0.003	0.44ab±0.005	0.43abc±0.002	0.44ab±0.006	0.43bc±0.01	0.45a0.002	0.44ab±0.002
Abdominal fat %	0.36±0.008	0.36±0.005	0.38±0.006	0.37±0.006	0.37±0.003	0.38±0.006	0.37±0.009	0.38±0.005

a-c Means in the same row with different letters, differ significantly ($p<0.05$).

Table 4: The chemical composition and quality of meat.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
Protein	19.26±0.27	19.56±0.36	19.43±0.25	19.31±0.37	19.49±0.36	18.98±0.22	18.97±0.39	19.20±0.47
Fat	4.00±0.12	3.87±0.07	3.98±0.13	4.19±0.17	4.08±0.17	4.45±0.02	4.27±0.26	4.30±0.27
Ash	1.22±0.02	1.23±0.02	1.19±0.04	1.23±0.03	1.22±0.04	1.17±0.01	1.21±0.01	1.18±0.01
Ph	6.83±0.04	6.86±0.03	6.79±0.04	6.80±0.01	6.79±0.03	6.75±0.03	6.84±0.03	6.78±0.03
Colors	0.24±0.01	0.21±0.01	0.24±0.01	0.22±0.01	0.22±0.01	0.25±0.01	0.23±0.01	0.23±0.01
Tenderness	2.78±0.12	2.68±0.12	2.84±0.15	2.75±0.13	2.76±0.08	2.77±0.17	2.74±0.11	2.86±0.07
WHC	5.75±0.10	5.80±0.12	5.64±0.13	5.87±0.10	5.60±0.11	5.92±0.10	5.64±0.13	5.64±0.14

Table 5: The effect of dietary treatments on nitrogen and ash retention %.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
Nitrogen retention %	53.63a±0.15	49.93bc±1.34	52.84ab±0.99	51.83abc±1.20	52.95ab±0.26	49.12c±0.60	52.00abc±1.29	52.59ab±0.45
Ash retention %	13.45b±1.3	4.58d±0.70	9.56bcd±1.20	5.89cd±0.88	11.20bc±1.50	21.74a±1.60	26.83a±0.99	27.16a±0.58

a-d Means in the same row with different letters, differ significantly ($p<0.05$).

percentage by about 6.89% compared to control group (49.93 VS 53.63%). Addition of AA and other additives except SS alone decreased the negative effect of heat stress on nitrogen retention percentage. These result agree with those obtained by Sahin and Kucuk (2001) who reported that utilization of dry matter, crude protein and an ether extract in Japanese quails kept at high ambient temperature is significantly decreased and that such negative effects were restored by vitamin C supplementation. Ostrowski (1981) showed that very short-term exposure of broilers to high temperatures (15 min at 42°C) results in reduced protein synthesis, depletion of essential and nonessential plasma free amino acids and increased plasma uric acid levels, possibly reflecting more active protein catabolism and reduced nitrogen retention. On the other hand, Temim *et al*. (1998) reported that muscle proteolysis tends to decrease when heat-stressed birds are fed high protein diets, which in turn may result in a reduced energetic cost of protein deposition.

One hypothesis to explain the increase nitrogen retention percentage as a result of addition AA or natural antioxidants is that heat stress increased Reactive Oxygen Species (ROS) leads to increase the protein oxidation and lost it in feces and the additives used scavenged the free radicals generated by heat stress. In this respect, Bottje *et al*. (2004), found that the increase in H_2O_2 production and the high protein oxidation were consistently observed in low feed efficiency duodenum, breast muscle and liver mitochondria compared to high feed efficiency birds. Iqbal *et al*. (2004) showed that If ROS did not removed by antioxidants, oxidation of critical structures in the mitochondria or cell or both, such as lipids, proteins and DNA, can lead to further inefficiencies that accentuate additional ROS generation. Abd El-Hakim *et al*. (2009) found that addition of *Curcuma longa* plus thyme to broiler diet significantly increased nitrogen retention by 13.25% compared to control diet. Also there were significant ($p<0.01$) differences between values of ash retention percentage. The heat stress treatment significantly decreased ash retention in HS birds by 65.94% compared to control diet. These results agree with those obtained by El-Husseiny and Creger (1981) who reported significantly lower rates of retention of minerals such as Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in broilers subjected to environmental stress. It was surprise that SS increased the ash retention by 61.63% compared to control diet. It is known that availability of sources of minerals were more when the elemental was in sulphate form. Also, the addition of SS to CL or CC increased ash retention percentage compared to them alone. SS alone did not increased the nitrogen retention, but increased ash retention percentage while AA increased both nitrogen and ash retention compared to HS group. Depending on data of performance (Table 2) and nitrogen retention (Table 5) we can hypothesis that under heat stress condition, we need to increase both nitrogen and ash retention. Grune *et al*. (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. On the other hand, some mineral such as Mn or Zn play role in antioxidants enzyme. For example, manganese is a crucial component of the metalloenzyme Mn Superoxide Dismutase (MnSOD). It has been determined that MnSOD functioning as a free radical scavenger is by far the most important Mn-containing enzyme (Luo *et al*., 1992). Also, One of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000).

Plasma parameters: The data in Table 6 indicated that there were significant ($p<0.01$) differences between values of plasma antioxidants capacity recorded by different experimental treatments. The birds fed CC+ SS recorded the highest value while the birds fed SS alone recorded the lowest. The addition of CL, CC alone or with SS increased plasma antioxidants capacity compared to HS group. The increase of plasma antioxidants by natural antioxidants supplementation have been reported by Ali *et al*. (2007) who found that addition of anise or thyme increased plasma antioxidants capacity of laying hens . The addition of SS to CL increased plasma antioxidants capacity much more than with CC and this may be due to difference of their active materials.

However, Prior *et al*. (2007) showed that the contribution of dietary phenolics to antioxidant activity *in vivo* might be lower than expected from *in vitro* tests. The increase in total antioxidant capacity in plasma or serum after consumption of antioxidants should indicate an absorption of the antioxidants and an improved *in vivo* antioxidant defense status (Cao *et al*., 1998). The conjugation of active material with sulphate may increase its antioxidants activity (Ali *et al*., 2007). On the other hand, Xenobiotic conjugation with sulphate is an important route for conversion of lipophilic xenobitics to more readily excreted polar metabolites (Jakoby, 1980). We believed that sulphate increase the activity of xenobiotics as antioxidants and if the bird does not need antioxidants, sulphate ion can eliminate it via urine or bile. There were significant ($p<0.01$) differences between values of plasma total lipid recorded by different treatments. The increase in total lipid in HS group could be explained by that birds tend to store much fat under heat stress. Ain Baziz *et al*. (1996) and Geraert *et al*. (1996) observed that fat deposition enhanced under chronic heat exposure conditions.

Addition of AA significantly decreased total lipids compared with HS group. There were insignificant differences in plasma total protein, albumin, globulin, cholesterol, AST and ALT values. However, Emadi and

Table 6: The effect of dietary treatments on plasma parameters.

Items	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
Antioxidants capacity mm/L	0.57bcd	0.49cd	0.50cd	0.59abc	0.67ab	0.41d	0.76a	0.65abc
	±0.02	±0.08	±0.07	±0.02	±0.05	±0.04	±0.01	0.005
Total lipids mg/dl	569.1c	747.9ab	596.2c	663.9abc	604.3bc	804.8a	558.2c	685abc
	±27.50	±30.0	±27.10	±19.5	±14.3	±29.1	±27.01	±26.0
Total protein g/dl	6.05	6.57	6.44	5.83	6.26	7.06	5.48	6.63
	±0.34	±0.39	±0.41	±0.48	±0.40	±0.32	±0.22	±0.50
Albumin g/dl	5.18	4.92	4.52	3.27	5.03	5.80	4.74	5.16
	±0.42	±0.55	±0.27	±0.33	±0.34	±0.40	±0.03	±0.34
Globulin g/dl	0.86	1.64	1.91	2.55	1.22	1.26	0.74	1.46
	±0.07	±0.18	±0.42	±0.40	±0.10	±0.24	±0.09	±0.25
Cholesterol mg/dl	162.2	158.4	165.1	157.4	155.5	165.6	169.0	161.1
	±7.10	±4.75	±7.01	±11.27	±7.99	±7.25	±6.44	±8.82
AST u/L	13.01	11.50	10.87	11.11	11.30	11.40	11.60	10.63
	±0.36	±0.49	±0.79	±0.46	±0.29	±0.58	±0.45	±0.49
ALT u/L	7.00	6.21	6.45	6.62	6.04	6.74	6.32	6.07
	±0.08	±0.42	±0.28	±0.10	±0.16	±0.41	±0.25	±0.28
Calcium mg/dl	11.48	11.47	11.56	11.27	12.87	12.07	11.66	12.09
	±0.59	±0.82	±0.55	±0.31	±0.26	±0.41	±0.48	±0.24
Phosphorus mg/dl	6.56	6.24	5.62	6.19	6.22	5.97	6.03	6.31
	±0.48	±0.53	±0.36	±0.35	±0.37	±0.29	±0.11	±0.13
Zinc µg/dL	21.96c	56.43ab	18.56c	39.39bc	70.83a	43.18bc	70.45a	19.31c
	±0.75	±2.50	±0.90	±1.84	±3.70	±2.12	±5.26	±0.65

a-d Means in the same row with different letters, differ significantly ($p<0.05$).

Kermanshahi (2007) fed broiler diets supplemented with CL powder at levels of (0.25, 0.5, 0.75%) from hatch to 49 d and concluded that supplementation of CL might have some positive effects on liver enzymes by reducing Alanine Amino Transferase (ALT) and alkaline phosphatase activities that directly or indirectly reflect a healthier liver status in the birds. While there were insignificant differences in plasma calcium and phosphorus, there were significant ($p\leq0.01$) differences in plasma zinc. The birds in HS group recorded values of plasma zinc were being higher by 156.9% compared to control diet. The increase in plasma zinc in HS group could be explained on basis that zinc is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000) so the birds increase level of zinc to protect the body from free radical. Davies (1995) showed that living organisms are able to adapt to oxidative stress by inducing the synthesis of antioxidant enzymes and damage removal/repair enzymes. The addition of AA decreased zinc level by 67.10% compared to HS group and this indicated that birds can use AA to eliminate the free radical and do not need to increase plasma antioxidants enzyme. The same results obtained with CC + SS and this also meaning that addition of SS raise the antioxidants activity of CC as well as AA.

Blood pH, PCV, Hgb: Table 7 shows the significant ($p<0.01$) differences between treatments on blood pH, PCV and Hgb values. Blood pH increased due to HS during course of HS as compared to the control group. AA and both natural antioxidants alone or with sulphate similarly led to numerically improved pH value compared to HS group. It is evident that the increase in blood pH value due to HS led to a further respiratory alkalosis and a reduction in the performance of chickens (Lin *et al*., 2006; Daghir, 2008). HS decreased Packed Cell Volume (PCV) and Hgb by 13.27% and 15.22%, respectively compared to control diet. The additives used in this study increased PCV and Hgb compared to HS group but not as a control diet. However, Attia *et al*. (2009) found that AA restore the negative effect of HS on Hgb.

Responses of SRBC's: HS had a negative effect on the responses to SRBCs at day 6 and 9 post-injection (Table 8). HS was also reported to cause a reduction in antibody production in young chickens (Zulkifi *et al*., 2000). Borges *et al*. (2004) concluded from their results that, in addition to the negative impact on broiler chicken performance, exposure to heat stress causes the immune response to be depressed, thus increasing the susceptibility of flocks to disease challenges. On the other hand, Donker *et al*. (1990) found that heat exposure did not reduce antibody production to SRBC's and Heller *et al*. (1979) even found significantly increased antibody titers to SRBC's following heat exposure. AA and other additives increased the responses to SRBC's. It was observed that the birds fed CC alone or with SS seemed to be greater immune response compared to other additives. These results in harmony with the data of performance and nitrogen retention.

Respiration rate and rectal temperature: Table 9 and 10 show the influence of different treatments and sampling time on RR and RT. The RR and RT increased

Table 7: The effect of dietary treatments on Blood pH, PCV and Hgb.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
pH	7.55d±0.003	7.68a±0.01	7.63bc±0.01	7.66ab±0.005	7.61c±0.008	7.68a±0.005	7.62bc±0.01	7.58d±0.01
PCV	33.90a±0.83	29.40c±1.06	31.63b±0.61	30.76bc±0.38	30.73bc±0.29	30.33bc±0.44	30.73bc±0.17	30.86bc±0.33
Hgb	9.00a±0.11	7.63b±0.18	7.96b±0.08	7.93b±0.26	8.13b±0.17	7.86b±0.14	8.10b±0.100	8.06b±0.17

a-d Means in the same row with different letters, differ significantly (p<0.05).

Table 8: The effect of dietary treatments on Post-injection responses to SRBCs at 3, 6 and 9 days.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
3- days	5.33±0.33	5.00±0.57	5.33±0.33	5.66±0.66	5.66±0.33	5.00±0.00	5.00±0.57	5.33±0.33
6-days	6.66±0.21	5.00±0.00	6.00±0.57	6.33±0.88	6.66±0.33	6.00±0.00	6.66±0.33	7.00±0.57
9-days	5.33±0.33	3.66±0.33	4.33±0.33	4.33±0.88	4.66±0.33	4.66±0.33	4.33±0.33	5.00±0.57

Table 9: The effect of dietary treatments on respiration rate during exposure to heat stress.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
During heat exposure								
2-weeks	54.00a±1.15	74.00c±0.57	64.66b±2.02	65.66b±0.88	66.00b±3.05	68.33b±0.88	66.00b±0.57	65.66b±2.18
5-weeks	55.00a±0.57	73.3c±0.88	63.00b±2.64	66.00b±1.00	67.00b±1.15	67.00b±0.57	66.33b±1.85	64.66b±0.88
8-weeks	54.66a±0.33	75.33c±0.88	65.33b±1.77	65.33b±0.88	67.00b±1.52	68.33b±1.20	64.66b±2.02	65.00b±1.52

a-b Means in the same row with different letters, differ significantly (p<0.05).

Table 10: The effect of dietary treatments on rectal temperature during exposure to heat stress.

Item	Control diet	HS	HS+AA	HS+CL	HS+CC	HS+SS	HS+CL+SS	HS+CC+SS
During heat exposure								
2-weeks	40.26a±0.03	43.07d±0.11	41.10b±0.12	41.40bc±0.28	41.37bc±0.18	41.60bc±0.33	41.75c±0.07	41.65bc±0.11
5-weeks	40.37a±0.01	43.17c±0.15	41.22b±0.04	41.74b±0.28	41.64b±0.16	41.77b±0.19	41.60b±0.10	41.67b±0.27
8-weeks	40.36a±0.005	43.28d±0.25	41.07b±0.19	41.49bc±0.19	41.07b±0.14	41.80c±0.19	41.52bc±0.12	41.58bc±0.12

a-c Means in the same row with different letters, differ significantly (p<0.05).

significantly (p<0.01) during HS at 2, 5 and 8 weeks compared to the other treatment. The additives used in this study significantly deceased the RR and RT compared to HS group. These results agree with those obtained by Attia *et al.* (2009).

Conclusion: The addition of CL, CC alone or with sulphate in the chicks diet can recover the negative effect of HS on performance, nitrogen retention, ash retention and plasma antioxidants capacity. To some extent these additives recover the negative effect on RR and RT. The CC plus SS seemed to be the best additive under the condition of this study.

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