Pak. J. Agri. Sci., Vol. 54(2), 443-450; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/17.5014
http://www.pakjas.com.pk

EFFECT OF DIETARY INCLUSION OF SODIUM BICARBONATE ON BLOOD PROFILE OF CAGED LAYERS DURING SUMMER

Ghulam Abbas, Sultan Mahmood*, Ahsan ul Haq and Haq Nawaz

Institute of Animal Sciences, University of Agriculture, Faisalabad, Pakistan
***Corresponding author's e-mail: drsultan2003@yahoo.com**

The intent of the study was to investigate the effect of dietary inclusion of sodium bicarbonate ($NaHCO_3$) on blood profile of caged layers during summer. One hundred sixty commercial layers were divided into 20 experimental units/replicates (8 layers/replicate), which were further allotted to five treatment groups (4 replicate/treatment). Five iso-nitrogenous (CP 17%) and iso-caloric (ME 2700 Kcal/Kg) diets (A, B, C, D and E) were prepared with or without addition of $NaHCO_3$. Diet A, was without $NaHCO_3$ and served as control whereas, diets B, C, D and E contained 0.5, 1.0, 1.5 and 2.0% $NaHCO_3$, respectively. These diets were fed to the experimental birds from 26th - 37th weeks of age (12 weeks). At the end of the experiment, blood samples of two birds from each replicate were collected for the analysis of blood profile. Results of the study revealed that serum glucose, white blood cells count, serum urea, plasma chlorides, serum cortisol and serum glutamic-oxaloacetic transaminase concentrations were found to be significantly ($P<0.05$) higher in control group, whereas, blood hemoglobin concentration, red blood cells count, plasma sodium, potassium, bicarbonate, serum total protein and serum albumen concentrations were significantly ($P<0.05$) higher in the birds fed diet containing 1% $NaHCO_3$. Whilst, Serum uric acid concentration was significantly ($P<0.05$) higher in those fed 2% $NaHCO_3$.However, packed cell volume, erythrocyte sedimentation rate, serum creatinine, alkaline phosphatase, plasma calcium,plasma phosphorus, serum globulin and serum glutamic pyruvate transaminase concentrations were not affected due to the dietary treatments. Serum cholesterol, triglycerides and low density lipoprotein concentration were significantly ($P<0.05$) decreased, whereas, serum high density lipoprotein concentration was found to be significantly ($P<0.05$) increased by dietary inclusion of $NaHCO_3$. Birds fed diet containing 1% $NaHCO_3$ showed significantly ($P<0.05$) higher concentration of estrogen, progesterone, T3 and T4 hormones. It was concluded that being economical and easy to handle, dietary inclusion of $NaHCO_3$ may be a better choice to reduce or at least ameliorate the harmful effects of heat stress on blood chemistry in caged layers reared in summer conditions.
Keywords: Heat stress, sodium bicarbonate, layers, serum cholestrol, hormones, lipoproteins

INTRODUCTION

Birds are able to maintain their body temperature within narrow limits (Khattak *et al.,* 2012). An increase in body temperature due to higher ambient temperature or excessive metabolic activities may cause irreversible thermoregulatory changes that could be harmful for the existence of birds (North and Bell, 1990) and may cause a change in physiological parameters such as rectal temperature, diseases, metabolic disorders and losses (Ahmad *et al.,* 2006; Anjum, 2000). As environmental temperature goes up, respiratory rate of birds rises causing higher losses of CO_2 that causes increase in blood pH and disturbs acid-base balance (Toyomizu *et al.,* 2005). Any change in acid base balance may cause alkalosis or acidosis, diverting the metabolic machinery used for homeostatic regulation rather than used for production (Carlson, 1997). Alteration in levels of CO_2 can cause disruption in blood pH. Growth performance of broilers was found to be the highest when blood pH was 7.28, whereas a decrease in growth was exhibited when pH values were greater than 7.30 or lower than 7.20 (Nelson and Cox, 2000).

Detrimental effect of heat stress on plasma electrolytes concentration has also been found to be similar in different species of birds such as in broilers (Mushtaq *et al.,* 2005), layers (Ghorbani and Fayazi, 2009) and quails (Keskin and Durgan, 1997). Heat stress is also known to disrupt status of reproductive hormones of laying birds, secreted by the hypothalamus and ovary (Elnagar *et al.,* 2010). Packed cell volume value of birds has shown an inverse relationship with high ambient temperature (Parker and Boone, 1971). Hypothermia (8°C) caused an increase in the hematocrits, whilst hyperthermia (30°C) caused a decrease in hematocrit value. High environmental temperature has also shown to cause a decrease in serum protein contents in birds (Anjum, 2000).

Heat stress has been observed to exert detrimental effects on various hemato-chemical parameters of birds (Borges *et al.*, 2004; Ekanayake *et al.,* 2004). However, various techniques are being used in birds such as addition/supplementation of various products in poultry rations to ameliorate the effects of heat stress. Supplementation of sodium bicarbonate

($NaHCO_3$) in water or feed is one of these practices, which can be used as an effort to combat heat stress in poultry birds (Ahmad, 1997; Mushtaq *et al.,* 2005). $NaHCO_3$ provides sodium and positively affects blood pH supplying bicarbonate ions (Ahmad *et al.,* 2006). Supplementation of sodium bicarbonate may help to maintain proper pH balance, eliminate acidosis and facilitate metabolic process, ensuring maximum growth and productivity (Ahmad *et al.,* 2005; Naseem *et al.*, 2005). Moreover, its use as dietary supplement may help rise in blood bicarbonate ion concentration, which can favor better performance of birds (Kaya *et al.,* 2004; Naseem *et al.,* 2005).

Information regarding potential beneficial effects of this substance on blood profile of layers during heat stress/summer is scanty, especially in the areas of Asiatic region.Based upon scientific information available, it can be envisaged that high environmental temperature can negatively influence blood profile in poultry birds. However, dietary addition of sodium bicarbonate during hot weather may improve bird's various blood parameters. Therefore, a project was initiated to check the response of dietary inclusion of sodium bicarbonate on blood profile in layers kept under hot weather conditions.

MATERIALS AND METHODS

One hundred sixty commercial layers of 24 weeks old were purchased from a commercial poultry farm. These birds were maintained in individual cages in a Poultry House of the Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad (Pakistan). These layers were divided in to 20 replicates (8 layers/ replicate). These replicates were further randomly allotted to five treatment groups (4 replicate/ treatment). Five diets *i.e.* A, B, C, D and E were formulated with or without addition of sodium bicarbonate. Diet A, was without sodium bicarbonate and served as control whereas, diets B, C, D, and E contained 0.5, 1.0, 1.5 and 2.0% sodium bicarbonate, respectively. All the diets were iso-nitrogenous (CP 17%) and iso-caloric (ME 2700 Kcal/kg) and were formulated according to the requirements prescribed by NRC (1994). These diets were fed to the experimental birds, from 26^{th}-37^{th} weeks of age (12 weeks). All the birds were maintained under similar management conditions like floor space, light, temperature, ventilation and relative humidity.

At the end of last week of the experimental period, blood samples from brachial vein (5 ml each) of two birds from each replicate were collected in separate screw top tubes for obtaining plasma and serum for the analysis of blood profile. Blood parameters like pH, HCO_3^- concentration (HCO_3^-, mmol/l), were determined by the Rapid Lab 248 pH/Blood Gas Analyzer (Kaya *et al.,* 2004). Plasma Na^+ and K^+ concentration were detected by using clinical flame photometer, whereas plasma calcium and potassium concentration were determined by atomic absorption spectrophotometer as described by AOAC (2010) using available commercial kit. White blood cells (WBCs) count (X 10^6/µl) and red blood cells (RBCs) count (X 10^3/µl) was determined by haemocytometer method (Despotis *et al.,* 1996). For erythrocytes sedimentation rate (ESR) determination, Westergren tube method (mm/hour) was used. Hemoglobin concentration (Hb) was determined using Drabkin's solution by Sahli's instrument and packed cells volume (PCV) was determined using microhaemocrite tubes by the method described by Coles (1991).

To quantify serum glucose, cholesterol, triglycerides and high density lipoprotein (HDL) level, Enzymatic Chod-Pap method as described by Anjum (2000) was used, whereas low density lipoproteins (LDL) was detected by the formula, i.e. (T_g/5 + HDL) – cholesterol. Total protein was assayed by biuret method and for the determination of albumin Bromocresol Green Redox Kit method was adopted whilst, serum globulin was determined by deducting albumin from total protein. Urea and uric acid in the serum was estimated by Enzymatic Kinetic method described in the Redox Kit following the UV method as used by Anjum (2000). Serum glutamic-pyruvate transaminase, serum glutamic-oxaloacetic transaminase, serum progesterone and serum estrogen were determined by the procedure followed by Anjum (2000). Whereas, blood serum cortisol was determined by the procedure described by Oelkers *et al.* (1992).

The data thus collected were subjected to statistical analysis for interpretation of results using completely randomized design (CRD) and differences in the treatment means were compared by the Least Significance Differences test (Steel *et al.,* 1997).

RESULTS

Finding of the present study revealed that glucose level, hemoglobin concentration and WBCs count in birds was significantly affected due to dietary inclusion of sodium bicarbonate in the layer diets (Table 1). Serum glucose and WBCs count was found to be lower in birds of group C as compared to those of control group. Birds of group C, which were fed diet containing 1% sodium bicarbonate, showed significantly higher ($P<0.05$) concentration of hemoglobin in their blood. However, red blood cells count, PCV and ESR values remained unaffected ($P<0.05$) due to the dietary treatments.

Mean values pertaining to serum urea, uric acid, creatinine and alkaline phosphatase concentration have been presented in Table 2. Serum creatinine concentration was found to be significantly ($P<0.05$) higher in the layers of control group whilst, serum uric acid concentration was found to be the significantly ($P<0.05$) higher in birds of group E (2% $NaHCO_3$). However, concentrations of serum creatinine and alkaline phosphatase were not affected due to the treatments.

Table 1. Effect of dietary inclusion of sodium bicarbonate on hematological profile ofcaged layers during summer.

Variables	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
ose (mg/dl)	212.70±17.84[a]	200.20±17.40[b]	195.30±5.91[bc]	186.60±5.88[c]	174.70±10.5[d]
(%)	33.00±3.56	32.50±2.46	32.70±2.99	32.00±2.16	32.70±2.63
mg/dl)	9.72±0.85 [b]	10.53±0.90[ab]	11.37±0.67[a]	10.58±1.16[ab]	10.75±1.32[ab]
(mm/hour)	3.93±0.29	3.75±0.44	3.70±1.04	3.73±0.62	3.70±0.52
s count (10^6/mm^3)	2.53±0.52	2.73±0.45	2.81±0.53	2.69±0.67	2.66±0.18
s count (10^3/mm^3)	26.50±1.21[a]	22.50±2.80[b]	24.00±2.73[ab]	25.00±2.51[ab]	26.20±1.00[a]

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation.

Table 2. Effect of dietary inclusion of sodium bicarbonate on serum metabolites of caged layers during summer.

Variables (mg/dl)	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
Serum urea	12.33±0.71[a]	8.96±1.31[b]	8.58±0.92[b]	9.57±1.02[b]	11.35±0.81[a]
Serum uric acid	5.90±0.70[b]	6.52±0.62[b]	6.62±0.45[b]	7.60±0.56[a]	7.80±0.78[a]
Serum creatinine	0.77±0.05	0.76±0.05	0.69±0.09	0.72±0.08	0.75±0.04
Alkaline Phosphatase	14.00±0.66	13.62±0.47	13.12±0.62	13.17±0.88	13.77±0.26

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation.

Table 3. Effect of dietary inclusion of sodium bicarbonate on serum proteins concentration of caged layers during summer

Variables (mg/dl)	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
Total protein	4.97±0.59[c]	6.13±0.65[a]	6.53±0.58[a]	6.02±0.50[ab]	5.05±0.49[bc]
Albumen	3.35±0.26[b]	3.82±0.26[ab]	3.90±0.53[a]	3.50±0.15[ab]	3.41±0.29[ab]
Globulin	1.62±0.18	2.31±0.32	2.63±0.62	2.52±0.15	1.63±0.13

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation.

Effect on serum total protein, albumen and globulin concentrations due to dietary inclusion of sodium bicarbonate are presented in Table 3. Inclusion of sodium bicarbonate exhibited a significant (P<0.05) increase in serum total protein and albumen concentration values of the experimental birds. Birds fed diets containing 1% sodium bicarbonate exhibited significantly (P<0.05) higher concentration of these proteins as compared to those of other groups. However, serum globulin contents of the birds remained unaffected (P>0.05) due to the dietary inclusion of sodium bicarbonate.

Mean values regarding plasma sodium,potassium,chloride, calcium, phosphorus, HCO_3 and serum pH are shown in Table 4. Plasma sodium level showed a significant (P<0.05) linear increase with increase in the level of dietary inclusion of sodium bicarbonate. Birds of group D (1.5% $NaHCO_3$) showed higher level of plasma sodium concentration. Serum potassium and bicarbonates also increased due to the dietary treatments. Birds of group B (0.5% $NaHCO_3$) exhibited higher plasma potassium concentration and birds of group E, which were fed diets containing 2% $NaHCO_3$ showed higher concentration of plasma bicarbonate. On the other hand birds fed diets containing sodium bicarbonate exhibited significantly (P<0.05) decreased levels of serum chlorides and serum pH values. Birds fed diet containing 1.5% $NaHCO_3$ (group D) depicted lower level of plasma pH. Plasma chloride concentration was linearly decreased with increasing the level of sodium bicarbonate in the diets of layers. However, plasma calcium and phosphorus levels remained unaffected (P>0.05) because of the sodium bicarbonate treatments.

Concentrations of serum cholesterol, serum triglyceride, serum high density lipoprotein (HDL) and serum low density lipoprotein (LDL) are presented in Table 5. Values of serum cholesterol, triglycerides and LDL were significantly (P<0.05) decreased by the dietary inclusion of sodium bicarbonate when compared to control group. The birds fed diets containing 1% $NaHCO_3$ exhibited lower concentration of these lipids as compared to those of other treated groups. Whereas, concentration of HDL was found to be increased (P<0.05) by dietary treatment and the birds fed

Table 4. Effect of dietary inclusion of sodium bicarbonate supplementation on plasma electrolytes and serum pH of caged layers during summer.

Variables	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
Plasma Sodium (mM/L)	132.70±9.35^{d}	141.30±8.70^{c}	149.60±11.62^{b}	155.20±9.10^{a}	154.70±8.69^{a}
Plasma potassium (mM/L)	3.92±0.31^{b}	4.97±0.20^{a}	4.87±0.22^{a}	4.86±0.54^{a}	4.10±0.26^{b}
Plasma chloride(mM/L)	136.46±1.84^{a}	118.21±5.95^{b}	102.11±3.84^{c}	94.63±3.26^{d}	77.46±3.07^{e}
Plasma HCO_3 (mM/L)	21.86±1.30^{d}	25.83±2.13^{c}	27.58±1.50^{b}	27.81±1.54^{b}	28.93±1.43^{a}
Plasma Calcium (mg/dl)	10.70±0.51	11.10±0.64	11.30±0.35	11.00±0.45	10.70±0.39
Plasma Phosphorus (mg/dl)	7.90±0.43	8.10±0.47	7.80±0.35	7.50±0.76	7.40±0.17
Serum pH	7.65±0.24^{a}	7.42±0.15^{ab}	7.38±0.22^{ab}	7.22±0.19^{b}	7.32±0.25^{b}

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation (SD).

Table 5. Effect of dietary inclusion of sodium bicarbonate on serum lipids profile of caged layers during summer.

Variables (mg/dl)	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
Serum cholesterol	161.25±9.20^{a}	149.50 ±2.71^{ab}	141.63 ±5.62^{b}	158.10±9.20^{a}	163.13±6.73^{a}
Serum triglycerid	296.00±27.0^{a}	226.00±19.0^{b}	163.00±8.00^{c}	203.00±20.0^{bc}	185.00±18.0^{bc}
Serum HDL	121.00±9.90^{b}	130.00±10.3^{ab}	138.00±13.7^{a}	141.00±4.50^{a}	154.00±9.10^{a}
Serum LDL	62.60±6.42^{a}	28.20±2.29^{b}	28.60±2.34^{b}	27.10±2.00^{b}	31.30±1.43^{b}

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation.

Table 6. Effect of dietary inclusion of sodium bicarbonate on serum hormones and liver enzymes of caged layers during summer.

Variables	Treatments				
	Control	0.5% $NaHCO_3$	1% $NaHCO_3$	1.5% $NaHCO_3$	2% $NaHCO_3$
Triiodothyronine (ng/ml)	2.87±0.16^{b}	3.07±0.15^{ab}	3.27±0.20^{a}	3.25±0.12^{a}	2.99±0.14^{b}
Thyroxine (ng/ml)	1.79±0.06^{c}	1.90±0.01^{c}	2.08±0.03^{a}	1.79±0.03^{c}	1.93±0.03^{b}
Cortisol (ng/ml)	71.25±2.22^{a}	69.25±2.75^{ab}	65.77±2.03^{b}	66.50±1.91^{b}	70.19±2.84^{a}
Estrogen (pg/ml)	143.30±8.00^{b}	168.60±5.34^{a}	171.30±3.42^{a}	168.30±4.82^{a}	166.80±9.80^{a}
Progesterone (ng/ml)	0.89±0.10^{b}	1.08±0.14^{a}	1.18±0.05^{a}	1.16±0.03^{a}	1.16±0.02^{a}
SGOT (U/L)	115.50±5.65^{a}	101.75±8.80^{b}	93.88±7.31^{c}	99.00±7.03^{bc}	98.13±6.95^{bc}
SGPT (U/L)	71.70±2.40	67.60±2.56	65.70±3.10	69.50±6.80	63.80±5.27

Values within the same row which have different superscripts are significantly (P<0.05) different. Data are average of four replicates ± standard deviation.

diets containing 1% sodium bicarbonate exhibited higher level of serum HDL in their blood.

Effects due to dietary inclusion of sodium bicarbonate on mean values of various serum hormones and liver enzymes of caged layers are presented in Table 6. Birds fed diets containing sodium bicarbonate exhibited increased concentration of T_3, T_4, estrogen and progesterone as compared to those of control group. The birds of group C (1% $NaHCO_3$) showed significantly (P<0.05) higher concentrations of estrogen, progesterone, T_3 and T_4 hormones. However, Serum glutamic pyruvate transaminase (SGPT) concentration was not affected (P>0.05) due to the dietary treatments. Serum glutamic-oxaloacetic transaminase (SGOT) and serum cortisol concentrations were significantly higher in birds of control group, which were fed diet without addition of sodium bicarbonate.

DISCUSSION

Dietary inclusion of sodium bicarbonate significantly (P<0.05) decreased serum glucose and WBCs count in the birds. As the ambient temperature was very high during the experimental period, therefore, the birds experienced a continuous heat stress. In response to combat this heat stress the birds in control group might have secreted higher level of hormones like glucocorticoid, adrenaline and noradrenaline (Yang *et al.,* 1992), which might have caused gluconeogenesis, ultimately leading to rise in blood glucose concentration. Decrease in blood glucose level and WBC

count of the birds of treated groups may probably be due to decrease in heat stress upon the birds as has been depicted in the results of the present study, where these birds have exhibited significantly lower rectal temperature as compared to those of control group. Khattak *et al.* (2012) have also reported a similar increase in WBCs count in the birds exposed to high ambient temperature as compared to those fed sodium bicarbonate containing diet at the same temperature.

The results of this study regarding serum glucose concentration are in line with the findings of Al-Hassani *et al.* (2001) and Ahmad *et al.* (2005) who observed a significant decrease in blood glucose level in birds fed diet containing sodium bicarbonate than those of exposed to heat stress (controls). However, , Yang *et al*. (1992) observed higher blood sugar contents at 23 and 28°C (223.6 and 221.7 mg/100ml) in broilers exposed to 12, 18, 23, 28 and 32°C temperature.

The packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and red blood cells (RBCs) count of all the experimental birds remained unaffected due to the inclusion of sodium bicarbonate in their diets. Conflicting results have so for been reported regarding the effect of dietary inclusion of sodium bicarbonate on packed cell volume of poultry birds. A significant decrease in packed cell volume has been observed by Oladele *et al.* (2001) in birds exposed to high environmental temperature. They attributed this increase to heat and nutritional stress, which impaired the synthesis of blood cells in the birds. Whereas,Mubarak and Sharkawy (1999),Al-Hassani *et al.* (2001) and Ahmad *et al.* (2005) have observed an increase in hematocrit values in birds treated with sodium bicarbonate and these findings are quite in contrast to the findings of present study. Similarly, contradictory to the findings of this study, Ekanayake *et al.* (2004) and, Mubarak and Sharkawy (1999) have reported increase in RBCs count in birds fed diets containing sodium bicarbonate.

Birds using diets containing sodium bicarbonate exhibited significantly higher hemoglobin concentration in their blood as compared to those of control group. Decline in hemoglobin concentration at higher environmental temperature in the birds of control group also coincides with the findings of Sahota and Gilani (1995), Yahav *et al.* (1997) and Vecerek *et al.* (2002). Addition of anti-stressors such as sodium bicarbonate,in drinking water of Leghorn and Matrouh hens markedly increased their hemoglobin concentration (%), even under heat stress conditions (Genedi, 2000). Therefore, increased hemoglobin concentration in $NaHCO_3$ treated groups may probably be due to the increased nutrient uptake and reduction in body temperature, which might have led to better physiological performance of the birds. Findings of the present study are in line with those reported by Ahmad *et al.* (2005) who observed increase in hemoglobin concentration in birds due to inclusion of sodium bicarbonate in their diet.

Dietary inclusion of different levels of sodium bicarbonate depicted a significant increase in plasma sodium, potassium and bicarbonate concentration of layers when compared to those fed diet without addition of sodium bicarbonate. These results are compatible to those observed byAhmad *et al.* (2006) as they reported an increase in plasma Na^+ and K^+ concentration in birds due to the supplementation of sodium bicarbonate in their diets. Similarly, Mushtaq *et al.* (2005) have also found an increase in concentration of these ions because of dietary addition of different levels of sodium. In consonant to the findings of present study, Keskin and Durgan (1997) and Squirea and Julian (2001) also found a significant increase in plasma bicarbonate level due to the inclusion of $NaHCO_3$ in the diet of birds exposed to heat stress. Serum pH was significantly reduced, nearer to the normal physiological value, by the dietary addition of sodium bicarbonate in heat stressed birds. These results are compatible to the findings of Ahmad *et al.* (2005) who reported that blood pH was decreased due to the supplementation of various sodium sources (sodium carbonate, sodium bicarbonate or sodium sulfate) in the diets of poultry birds. Similarly Mushtaq *et al.* (2005) reported that blood pH was significantly decreased because of increasing dietary addition of sodium level (0.20% vs 0.25%). Khattak*et al.* (2012) have also observed a decrease in blood pH (8.04) in broilers kept at 38-40°C as compared to those fed sodium bicarbonate containing diet (8.34 pH), at the same temperature. In the present study, decrease in plasma chloride concentration in the birds fed different levels of sodium bicarbonate may be attributed to increased level of sodium in their blood. These findings are compatible to those observed by Ahmad *et al.* (2006) who reported that blood Cl^- concentration was decreased by the supplementation of various sodium sources (sodium carbonate, sodium bicarbonate or sodium sulfate) in the diets of poultry birds.

Birds using diets containing sodium bicarbonate exhibited significantly lower serum lipid concentration profile *i.e.* low density lipoproteins(LDL), cholesterol and triglycerides level in their serum**,** when compared to those of control group. A probable explanation of decrease in the concentrations of these parameters may be that $NaHCO_3$ might have stimulated the synthesis of bile acids from cholesterol, leading to decreased concentration of serum cholesterol in these birds (Naviglio *et al.*, 2011). Fasting in heat stressed birds might have increased the blood cholesterol level by mobilizing fat through gluconeogenesis which ultimately resulted in increased blood cholesterol level (Hevia and Vinsek, 1979). High serum cholesterol concentration in birds kept at high temperature has also been reported by Takahashi *et al*. (1991) and Sahota *et al*. (1993). Therefore, another possible reason for reduction in

serum lipids may be the inhibition of enzyme "squalene epoxidase" due to the treatment, which is essential for synthesis of cholesterol (Angelovicova, 1997).

Hypothalamus and pituitary receive stimulus of heat stress and in turn causes a decline in the secretions of Tri-iodothyronine and Thyroxin (Anjum, 2000), whereas the higher concentration of these hormones results in improved performance of birds. As dietary inclusion of sodium bicarbonate in the results of present study has shown to reduce heat stress. Hence, dietary addition of bicarbonate might have caused an increase in concentration of these hormones in the blood of treated birds resulting in reduction of heat stress. These results are also compatible to the findings of Remus (2001).

Response of birds due to dietary inclusion of sodium bicarbonate with respect to growth hormones has not been studied much. However, contrary to the results of present study, Hassan *et al.* (2011) have observed a significant ($P<0.05$) decrease in plasma T_3 and T_4 hormones concentration in laying birds supplementing diet with sodium bicarbonate. Similar findings have been reported by Attlla *et al.* (2002) who found that supplementing of $NaHCO_3$ and KCl in drinking water, decreased concentration of T_3 hormone in hens exposed to high environmental temperature (34°C) for 4 continuous hours daily followed by a normal temperature (22 to 24°C) throughout three months experimental period. Genedi (2000) found that adding $NaHCO_3$ to drinking water for heat stressed hens of Matrouh strain, reduced plasma triiodothyronine hormone (T_3); however, its effect was found to be non- significant in Leghorn hens. Findings of Badran (2003) have also shown that the level of plasma T_3 hormone in laying hens remained unaffected due to the addition of different levels of sodium bicarbonate (2, 3 and 4%).

Heat stress caused an increase in the concentration of serum corticosterone hormone in birds, however, dietary inclusion of sodium bicarbonate helped in reducing the level of this hormone. Reduction in serum corticosterone concentration in the layers fed $NaHCO_3$ treated diets also corresponds to decrease in their rectal temperature, as has been depicted in the results of this study. The highest level of corticosterone hormone was observed in the birds of control group, which were fed diet without addition of sodium bicarbonate. An increase in corticosterone level of birds, with increase in environmental temperature has also been reported by Sahin *et al.* (2002) and Tuekam *et al.* (1996).

The birds fed diets containing sodium bicarbonate exhibited higher serum estrogen and progesterone concentration when compared to those of control group. When ambient temperature goes beyond thermo-neutral zone, chemical reactions speed up in the body, heat is generated and body temperature of birds rises (North and Bell, 1990) which probably may have depressed ovarian functions, posing hindrance against the release and normal synthesis of these hormone**s.** It has also been observed that under such conditions sodium bicarbonate can be used as a buffering agent to ameliorate the effect of heat stress (Whiting *et al.,* 1991). These findings are compatible with those observed by Anjum (2000) who reported a significant decrease in progesterone concentration in layers exposed to heat stress.

The layers of control group depicted significantly higher concentration of serum glutamic-oxaloacetic transaminase (SGOT). Heat stress has shown to increase plasma cortisol concentration (Sahin, *et al.*, 2002) in birds. Increase in cortisol concentration may cause an increase in catabolic effect in liver, which exerts maximum stress on it, leading to an increase in the concentration of SGOT in blood serum of the birds exposed to heat stress (Anjum, 2000). However, birds fed diets containing sodium bicarbonate exhibited a significant reduction in SGOT concentration. A possible explanation of this fact may probably be the reduction in body temperature of birds fed diets containing sodium bicarbonate, which might have reduced stress on their liver and hence led to a decrease in SGOT concentration in these birds. These findings are compatible to those observed by Ahmad *et al.* (2005) who reported that dietary inclusion of sodium bicarbonate can decrease body temperature of heat stressed birds. In contrast to the results of present study, Ozbey *et al.* (2004) observed that blood SGOT concentration was not affected due to heat stress in quails. Difference in results of these studies, however, may probably be due to difference in ambient temperature maintained or difference in species of the birds (layers vs quails), used in these studies. However, concentration of Serum Glutamic Pyruvic Transaminase (SGPT) remained unaffected in the birds due to the addition of sodium bicarbonate in their diets.

***Conclusions*:**Based upon the results of the present study it can be concluded that dietary inclusion of sodium bicarbonate had beneficial effects on most of the blood parameters studied, such as blood hematology, plasma electrolytes, serum proteins, serum metabolites, serum hormones and liver enzymes of caged White Leghorn layers reared under hot climatic conditions. All these blood parameters are helpful in efficient production of poultry birds. Therefore, dietary inclusion of $NaHCO_3$ may be a quite useful technique to reduce or at least ameliorate the harmful effects of heat stress in caged layers reared in summer conditions.

***Acknowledgement*:** Financial support from Higher Education Commission, Islamabad, is highly acknowledged. The authors are also grateful to Professor Dr. Zafar Iqbal, Dean Faculty of Veterinary Sciences, UAF for providing Poultry Shed for the execution of this project.

REFRENCES

Ahmad, M.M and M. Sarwar. 2006. Dietary electrolyte balance implications in heat stressed broilers. World's Poult. Sci. J. 62:638-653.

Ahmad, T., M. Sarwar, M. Nisa, A. Haq and Z. Hasan. 2005. Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. Anim. Feed Sci. Technol. 120:277-298.

Ahmad, R. 1997. Growth performance and electrolyte balance of broiler as affected by two sources of sodium. M. Sc. (Hons.) Thesis, Department of Poultry Science, University of Agriculture, Faisalabad, Pakistan.

Al-Hassani, D.H., H.J. Al-Daraji and I.A. Abdul-Hassan. 2001. Effect of dietary sodium bicarbonate on some physiological parameters in Hisex Brown layers reared under high environmental temperature. Recent Advances in Animal Nutrition held in Australia' 13:32A.

Anjum, M.S. 2000. Productive performance and physiological behavior of White Leghorn caged layers under different heat combating practices during summer. PhD. Thesis, Department of Poultry Science. University of Agriculture, Faisalabad, Pakistan.

Angelovicova, M. 1997. Reduced cholesterol content in market eggs as influenced by diets with Amaranthus (*Amaranthus hypochondriacus*). Achta-Zootechnica 53:81-87.

AOAC. 2010. Official Methods of Analysis. Association of official analytical chemists. EUA 19th Ed., Washington DC, USA.

Attlla, A.A., M.R. Fatma, S.M. Salwa and M.G. Doaa. 2002. Effect of some electrolytes on the performance and some physiological parameters of laying hens.Egypt. Poult. Sci. 22:219-233.

Badran, A.M.M. 2003. Effect of sodium bicarbonate supplementation on laying performance and physiological parameters during hot weather. M.Sc. Thesis Faculty of Agriculture, Cairo University, Egypt.

Carlson, G.P. 1997. Fluid, electrolyte and acid-base balance. In: J.J. Kaneko, J.W. Harvey and M.L. Bruss (eds.), Clinical Biochemistry of Domestic Animals, 5th Ed.Academic Press, Boston; pp.485-516.

Coles, E.H. 1991. Veterinary Clinical Pathology, 4th Ed. W.B. Sanuders Company, London; pp.280-293.

Despotis, G.J., A. Alsofier, C. Wltoque, T.N. Zoys, L.T. Goodnough, S.A. Santoro, K.M. Kater, P. Bames and D.G. Lappas. 1996. Evaluation of complete blood count results from a new, on site hemocytometer compared with laboratory based hemocytometer. Crit. Care Med. 24:1163-1167.

Ekanayake, S., S.S. Silva, N. Priyankarage, U.T. Herath, M.U. Jayasekara, N.U. Horadagoda, P. Abeynayake and S.P. Gunaratne. 2004. Effect of excess sodium in feed on haematological parameters and plasma sodium level in broiler chickens. Br. Poult. Sci. 1:53-54.

Elnagar, S.A., S.E. Scheideler and M.M. Beck. 2010. Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal polypeptide-immunoreactive cells in hypothalamus in the heat stress-induced or chemically induced hypothyroid laying hen. Poult. Sci. 89:2001-2009.

Genedi, D.M.M. 2000. The role of some anti- stressors on layer performance during hot climate conditions. M.Sc. Thesis Faculty of Agriculture, Cairo University, Egypt.

Ghorbani, M.R. and J. Fayazi. 2009. Effects of dietary sodium bicarbonate and rearing system on laying hens performance, egg quality and plasma cations reared under chronic heat stress. Biol. Sci. Res. J. 4:562-565.

Hassan, M.S.H., O.A. El-Sayed and M.M. Namera. 2011. Effect of dietary sodium bicarbonate and potassium chloride supplementation on acid-base balance, plasma electrolytes and aldosterone hormone of golden montazah hens under hot climate condition. Egypt. Poult. Sci. 31:285-303.

Hevia, P. and W.J. Vinsek. 1979. Dietary protein and plasma cholesterol in chickens. J. Nut. 109:32-38.

Kaya, I., B. Karademir and O. Ukar. 2004. The effects of diet supplemented with sodium bicarbonate upon blood pH, blood gases and eggshell quality in laying geese. Vet. Med. Czech. 49:201-206.

Keskin, E. and Z. Durgan. 1997. Effects of supplemental $NaHCO_3$, KCl, $CaCl_2$, NH_4Cl and $CaSO_4$ on acid base balance, weight gain and feed intake in Japanese quails exposed to constant chronic heat stress. Pak. Vet. J. 17:60-64.

Khattak, F.M., T. Acamovic, N. Sparks, T.N. Pasha, M.H. Hussain, M. Joiya and Z. Ali. 2012. Comparative efficacy of different supplements used to reduce heat stress in broilers. Pak. J. Zool. 44:31-41.

Mubarak, M. and A.A. Sharkawy. 1999. Toxopathology of gout induced in laying pullets by sodium bicarbonate toxicity. Environ. Toxicol. Pharmacol. 7:227-236.

Mushtaq, T., M. Sarwar, H. Nawaz, M.A. Mirza and T. Ahmad. 2005. Effect and interactions of dietary sodium and chloride on broiler starter performance (hatching to twenty-eight days of age) under subtropical summer conditions. Poult. Sci. 84:1716-1722.

Naseem, M.T., S. Naseem, M. Younus, Z.I. Chauhdary, A. Ghafoor, A. Aslam and S. Akhter. 2005. Effect of potassium chloride and sodium bicarbonate supplementation on thermo-tolerance of broilers exposed to heat stress. Int. J. Poult. Sci. 4:891-895.

Naviglio, D., M. Gallo, L.L. Grottaglie, C. Scala, L. Ferrara and A. Santini. 2011. Determination of cholesterol in Italian chicken eggs. J. Food Chem.11:3-7.

North, M.O. and D.D. Bell. 1990. "Egg quality" Commercial Chicken Production Manual 4th Ed. Van Nostrand Rinolt, New York, NY.

Nelson, D.L., and M.M. Cox. 2000. Lehninger Principles of Biochemistry. Worth Publishers, New York, NY.

NRC. 1994. Nutrient requirements of poultry, 9th Ed. National Academy Press, Washington DC, USA.

Oelkers, W., S. Diederich and V. Bähr. 1992. Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity, and aldosterone. J. Clin. Endocrinol. Metab. 75:259-264.

Oladele, S.B., S. Ogundipc, J.O. Ayo and K.A.N. Esievo. 2001. Effects of season and sex on packed cell volume, haemoglobin and total proteins of indigenous pigeons in Zaria, Northern Nigeria. Veterinarski Arhiv. 71:277-286.

Ozbey, O., N. Yildiz, M.H. Aysöndü and O. Ozmen. 2004. The effects of high temperature on blood serum parameters and the egg productivity characteristics of Japanese quails (*Coturnix coturnix japonica*). Int. J. Poult. Sci. 3:485-489.

Parker, J.T. and M.A. Boone. 1971. Thermal stress effects on certain blood characteristics of adult male turkeys. Poult. Sci. 50:1287-1295.

Remus, J. 2001. Betaine for increased breast meat yield in turkey. World Poultry 17:14-15.

Sahin, K., O. Kucuk, N. Sahin and M. Sari. 2001. Effect of vitamin C and vitamin E on lipid peroxidation b status, some serum hormone, metabolite and mineral concentrations of Japanese quails reared under heat stress (34°C). Int. J. Vitam. Nutr. Res. 71:27-31.

Sahota, A.W., M.F. Ullah, A.H. Gilani and M.D. Ahmad. 1993. Effect of ascorbic acid supplementation on the performance of Lyallpur Silver Black and White Leghron chicks exposed to heat stress. Pak. Vet. J. 12:28-31.

Squires, E.J. and R.J. Julian. 2001. The effect of dietary chloride and bicarbonate on blood pH, haematological variables, pulmonary hypertension and ascites in broiler chickens. Br. Poult. Sci. 42:207-212.

Takahashi, K. and Y. Akiba. 2002. Effect of oral administration of Diakur (a glucose and electrolyte additive) on growth and some physiological responses in broilers reared in a high temperature environment. Asian-Aust. J. Anim. Sci.15:1341-1347.

Toyomizu, M., M. Ueda, S. Sato, Y. Seki, K. Sato and Y. Akiba. 2005. Cold-induced mitochondrial uncoupling and expression of chicken UCP and ANT mRNA in chicken skeletal muscles. Federation of European Biochemical Societies Letters 529:313-318.

Tuekam, T.D., R.D. Miles and G.D. Butcher. 1994. Performance and humoral response in heat stressed broilers fed an ascorbic acid supplemented diet. J. Applied Anim. Res. 6:121-130.

Whiting, T.S., L.S. Andrews, M.H. Adams and L. Stamps. 1991. Effects of sodium bicarbonate and potassium chloride drinking water supplementation. 2. Meat and carcass characteristics of broilers grown under thermoneutral and cyclic heat-stress conditions. Poult. Sci. 70:60-66.

Yang, Q.M., Q.W. Mu., Z.H. Yu and H. Lin. 1992. A study of influence of environmental temperature on some biochemical indices in serum of broilers. J. Shandong Agri. Univ. 23:363-367.

.