Pakistan J. Zool., vol. 44(1), pp. 31-41, 2012.

Comparative Efficacy of Different Supplements Used to Reduce Heat Stress in Broilers

Farina Malik Khattak,[1] Thomas Acamovic,[1] Nicholas Sparks, [1] Talat Naseer Pasha,[2] Mussarat Hussain Joiya,[2] Zafar Hayat[2] and Zulifqar Ali[3]

[1]Avian Science and Research Centre, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG Scotland.

[2]Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan

[3]Big Bird Pvt. Ltd, Lahore, Pakistan

Abstract.- A study was conducted to compare the effects of supplemental betaine (1200 mg/kg), vitamin C (VC, 200 mg/kg), vitamin E (VE, 300 mg/kg) and sodium bicarbonate ($NaHCO_3$, 2 g/kg) on the performance of 250 broilers during hot (38-40°C) and humid (40-93%) environmental conditions. Body weight and feed intake was recorded weekly. On day 35 pH, anti ND virus titre (HA, HI), Na, K and Cl concentrations and total leukocytic counts (TLC) were determined blood samples. All diets containing feed supplements gave better ($P<0.05$) weight gain and FCR than control. Lowest blood pH ($P<0.05$) was observed in birds fed supplemental $NaHCO_3$ compared to all other treatment groups. Serum Na and K levels were higher ($P<0.05$) in control group than all other supplemental groups. Maximum TLC ($P<0.05$) were observed in birds fed control diet compared to lower levels in birds fed diets containing $NaHCO_3$. A lowest ($P<0.05$) mortality was observed with betaine (3.3%), whereas, highest mortality (33%) was in control group. This study showed that appropriate feed supplements attenuate the decline in performance caused by heat stress. Among different diet supplements, betaine and $NaHCO_3$ offered better protection against heat–stress-related depression in performance of broilers.

Key words: Heat stress, broilers, vitamins, betaine, sodium bicarbonate.

INTRODUCTION

Birds are homeotherms, having the ability to maintain their body temperature within a narrow range. An increase in body temperature above the regulated range, as a result of exposure to environmental extremes and/or excessive metabolic heat production, may initiate a cascade of irreversible thermoregulatory events that could be lethal for the bird (North and Bell, 1990). High ambient temperature is very disruptive for broiler chickens and reduces feed intake (Smith and Teeter, 1987), weight gain, carcass weights, and abdominal fat (Pourreza and Edriss, 1992) and increases mortality (Belay and Teeter, 1996).

The commercial broiler industry in Pakistan, and other countries with hot climates, faces a great challenge during summer due to high environmental temperature. Heat stress adversely affects the performance of broilers which is commercially detrimental to producers. Therefore to avoid excessive losses due to heat stress many broiler farmers discontinue rearing of birds during the hot months.

Several methods have been proposed for reducing heat stress effects in poultry including nutritional methods (Daghir, 1995). Among the most important proposed nutritional methods are restricted feeding during the hottest hours of the day, to alter dietary levels of energy, protein or amino acids (Daghir, 1995; Han and Baker, 1991), to use fat in the diet (Daghir, 1995), or to supplement the diets with different additives such as vitamin C (Kutlu and Forbes, 1993; Whitehead and Keller, 2003), ammonium chloride (Smith and Teeter, 1993), potassium chloride (Deyhim *et al.*, 1990; Smith and Teeter, 1992, 1993), sodium bicarbonate (Balnave and Gorman, 1993; Hayat *et al.*, 1999). A number of studies have shown beneficial responses to different feed additives independently however, no studies have been conducted to directly compare the effects of different feed supplements at the recommended concentrations suggested by other authors. The

* Corresponding author: drfarinamk@yahoo.co.uk
0030-9923/2012/0001-0031 $ 8.00/0

present experiment was therefore conducted to compare the suitability and efficacy of different dietary supplements such as vitamin C (VC), vitamin E (VE), betaine and sodium bicarbonate ($NaHCO_3$) in the relief of heat stress in broilers.

MATERIALS AND METHODS

Starter and finisher diets were formulated using corn (maize), rice, rapeseed meal and soya meal (Table I). These diets contained the nutrient concentrations recommended by NRC (1994) except ME and CP which were used at levels lower than that of NRC recommendations. The selected levels are those which are used in the poultry feed industry in Pakistan. A commercial ration with no additional feed supplement was treated as the control (group A), whereas groups B, C, D and E contained 1200, 200, 300 2000 mg/kg of betaine, VC (ascorbyl palmitate), VE (α-tocopherol acetate) and $NaHCO_3$ in feed respectively. Betaine, VC and VE were purchased from Danisco and $NaHCO_3$ was purchased from ICI Pakistan. Dietary electrolyte balance (DEB = the sum of the dietary concentration of Na^+K-Cl) of the broiler starter diet was 222.4 and for finisher diet was 214.2. Addition of $NaHCO_3$ at 2g/kg of diets, resulted in a DEB 0f 261.5 and 256.7mEq/kg for broiler starter and finisher, respectively. The feed was pelleted and crumbed (2 mm). Maximum pelletting temperature was not more than 65°C. These experimental diets were fed to the birds from day 1 to day 35.

The trial was conducted at the Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore. Two hundred and fifty, 1-d old Hubbard x Hubbard classic broilers of mix sex, were purchased from a local hatchery. Birds were housed in open shed. No mechanical mean was used to reduce temperature or manipulate humidity. Birds were reared on natural environmental conditions throughout the experimental period. The study was conducted during the hot and humid summer months of June and July in Pakistan. Daily temperature and humidity was recorded (Table II) twice a day in the morning and (8am) and afternoon (3pm). The trial was conducted using a completely randomized experimental design, with five experimental diets, five replicates with ten birds in each. Birds were reared in floor pens with dimension of about 1.5 × 1.5 m. Water and feed were offered *ad libitum* and 24 hour lighting programme was adapted. Chicks were vaccinated against Newcastle Disease at day 7 and 27 days of age and Infectious Bursal disease vaccine at day 11 and 22 of age, administered through the drinking water.

Table I.- Feed Composition of basal starter and finisher diets.

Ingredient name	Basal starter (Crumbs)	Basal finisher (Crumbs)
Corn	39.2	31.5
Rice	20	20
Wheat	---	8.0
Rice polishing	1.5	5.74
Soya meal	17	13.62
Canola meal	9.76	9.99
Sunflower meal	3.62	2.08
Rapeseed meal	2.5	2.0
Fish meal	0	1.0
Dicalcium phosphate	1.95	1.29
Molasses	2.5	2.92
Vitamin mineral premix	1.0	1.0
Lime Stone	0.66	0.5
Sodium chloride	0.20	0.164
DL methionine	0.21	0.187
Total	1000	1000

Vitamin and mineral premix contributed the following nutrients per kilogram of complete feed.
Starter vitamins: Vitamin A 11000 IU; vitamin D3 3000 IU; vitamin E 35mg; vitamin K (menadione) 0.75mg; thiamine 4.9mg; riboflavin 7.2mg; pyridoxine 4.9mg; cyanocobalamine 0.015mg; niacin 69.3mg; pantohotenic acid 16.6mg; choline 350mg; folic acid 1.425 mg; biotin 0.14 mg; copper 7.5mg; iron 42mg; manganese 55mg; zinc 54mg; selenium 0.35mg; iodine 1.0mg; antioxidant 125mg; salinomycine 60mg; zinc
Finisher vitamins: Vitamin A 8900 IU; vitamin D3 2400 IU; vitamin E 28mg; vitamin K (menadione) 0.60 mg; thiamine 3.92mg; riboflavin 5.8mg; pyridoxine 3.9mg; cyanocobalamine 0.012mg; niacin 55.5mg; pantohotenic acid 13.3mg; choline 300 mg; folic acid 1.15 mg; biotin 0.12 mg; copper 7.5 mg; iron 42 mg; manganese 55mg; zinc 54mg; selenium 0.35mg; iodine 1.0mg; antioxidant 125mg; salinomycine 60mg; zinc

Feed intake and body weight was measured on a weekly basis. These data were used to calculate weight gain and feed conversion ratio. Mortality was also recorded throughout the study period. At the end of experiment, blood samples from two birds/ replicate were taken and kept at room temperature for 3 hours and then at 4°C overnight.

Each blood sample was centrifuged for 10 minute at 2000 rpm. Serum was removed and stored in sterile plastic tubs until used for mineral and titre analysis. Blood samples were used to determine pH using a pH meter. Anti-Newcastle disease virus titre (HA, HI) was determined as described by Rubbani *et al.* (2001), Na and K concentrations by flame photometry, Cl by titrimetric methods (Lacroix *et al.*, 1970), and total leukocytic counts (TLC) by the method as described by Benjamin (1986).

Table II.- Minimum to maximum room temperature and humidity range during 5 week experimental period.

Week	Ambient temperature range (Min to Max °C)	Humidity range (Min to Max %)
1	35-40	40-60
2	30-39	40-62
3	35-41	45-65
4	32-40	70-93
5	32-35	75-85

The data thus collected was analyzed statistically by one way analysis of variance (Minitab release 11). Difference in means if found were compared using Tukey LSD.

RESULTS

In the present study the birds encountered naturally high ambient temperatures (minimum 30ºC and maximum 41ºC) and the average % RH (minimum 40 and maximum 93) throughout the experimental period for 10h daily.

Body weight gain

The overall body weights were lower than the target weight range (1.7 to 2 kg) for Hubbard Classic at day 35 clearly indicated effects of heat stress. The birds fed on diets containing the dietary additives gave better ($p<0.05$) weight gains (Table III). When weight gain data was analysed on a weekly basis (Fig. 1) the data clearly indicated that on d-28 the lowest ($p<0.05$) weight gains were in birds fed the control diet compared to birds fed on diet E containing $NaHCO_3$. However, at the end of the trial at d-35 all birds fed on supplemented diets had better ($p<0.05$) weight gains as compared to the control group (A) where no supplement was added.

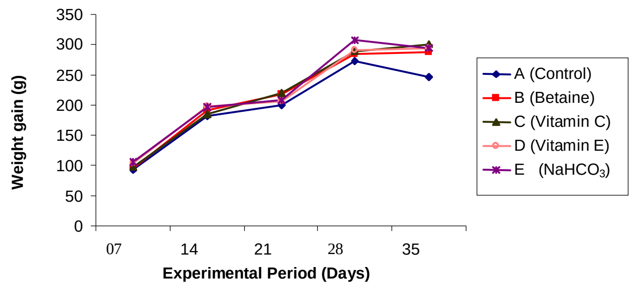

Fig. 1. Weekly weight gain of birds fed experimental diets.

Table III.- Weight gain, feed consumption and FCR of birds fed on different experimental rations over a five week experimental period

Treatment	Weight gain (g/chick)	Feed intake (g/chick)	FCR
A (Control)	980[a]	2107.4[b]	2.15[a]
B (Betaine)	1167[b]	1900.8[c]	1.63[b]
C (Vitamin C)	1187[b]	1974.7[c]	1.66[b]
D (Vitamin E)	1084.3[b]	2027.6[a]	1.87[b]
E ($NaHCO_3$)	1140.1[b]	1959.9[c]	1.71[b]

[a,b]Means within a column lacking common superscript differ significantly ($P<0.05$) separated by least significant difference test

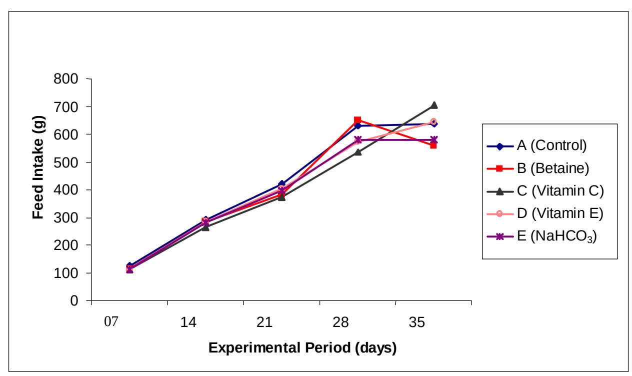

Fig. 2. Weekly feed intake of birds fed different experimental diets

The overall feed intake data indicated that all birds fed on diets containing VE and no supplements (control group A) had higher ($p<0.05$) feed intake compared to other treatment groups (Table IV). A prominent increase ($p<0.05$) in feed intake was recorded at d-28 where birds fed diets containing betaine and control diet compared to reduced ($p<0.05$) feed consumption in birds fed diet containing VC & $NaHCO_3$ (Fig. 2). However, at d-35 of the trial feed consumption of birds fed on VC increased ($p<0.05$) compared to decreased ($p<0.05$) feed intake in birds fed diet containing $NaHCO_3$ and betaine.

Table IV.- Determined feed composition of basal experimental starter and finisher diets.

Ingredient name	Starter (Crumbs)	Finisher (Crumbs)
Metabolizable energy (MJ/kg)	2775	2875
Crude protein (g/kg)	19.2	18.6
Fat (%)	2.54	3.43
Linoleic acid (%)	1.4	1.5
Fibre (%)	4.1	4.26
Ash (%)	5.15	5.6
Calcium (%)	1.0	0.9
Phosphorus Available (%)	0.45	0.40
Potassium (%)	0.8	0.78
Chloride (%)	0.23	0.21
Sodium (%)	0.19	0.17
Lysine (%)	1.18	1.07
Methionine (%)	0.55	0.50
Methionine + cystine (%)	0.88	0.82

The overall FCR data indicated that all birds fed on diets containing supplemental nutrients had consistently lower and better ($P<0.05$) FCR compared to the control group (Table III). Whereas, the weekly trend in FCR (Fig. 3), indicated that highest and poor ($p<0.05$) FCR was observed in control group birds where no supplemental nutrients were used to reduce heat stress through out the experimental period. At d-21 birds fed supplemental betaine and VC gave better ($p<0.05$) FCR. However, there was no difference ($p<0.05$) in FCR of supplemental groups at d-28. However, among birds fed different feed supplements, lowest ($p<0.05$) FCR was observed in birds fed on diets containing betaine, and $NaHCO_3$.

Blood pH

All the blood samples showed a relatively high pH and highest ($p<0.05$) alkalinity (pH=8.34) was observed in birds fed on the unsupplemented control group A compared to lowest ($p<0.05$) alkalinity (pH=8.04) in group E where birds were fed diet containing supplemental $NaHCO_3$ (Table V).

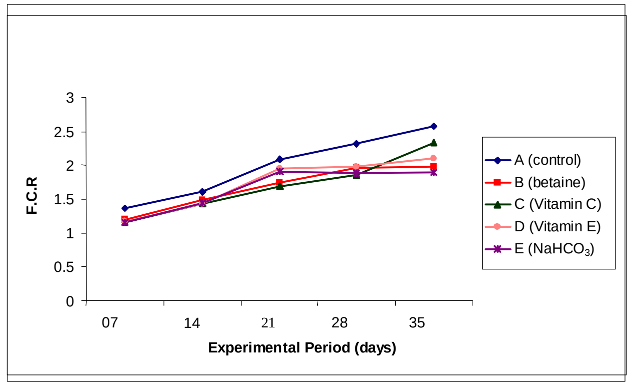

Fig. 3. Feed Conversion ratio (FCR) of birds fed different experimental diets

Table V.- Anti-New Castle Disease Virus titers, blood pH and mortality % of birds fed on experimental rations at the end of the experiment.

Group	Blood pH	Total leucocytic count (µl)	New castle disease titers	Mortality (%)
A (Control)	8.34^a	$3.2\times10^{4\,a}$	2.33^a	33^a
B (Betaine)	8.14^a	$1.5\times10^{4\,b}$	2.78^a	3.3^b
C (Vitamin C)	8.13^a	$2.1\times10^{4\,c}$	2.98^a	16^d
D (Vitamin E)	8.22^a	$2.2\times10^{4\,c}$	2.18^a	23^e
E ($NaHCO_3$)	8.04^b	$1.8\times10^{4\,b}$	3.91^b	6.6^c

[a–e]Means within a column lacking common superscript differ significantly ($P<0.05$) separated by least significant difference test.

Total leukocytic count

Total leukocytic counts (TLC) were maximum ($P<0.05$) in the blood samples from control group A compared to minimum ($P<0.05$) TLC in the birds fed on diet containing betaine and $NaHCO_3$ (Table V). It was interesting to note that TLC count of birds fed on VC and VE were higher ($p<0.05$) than other supplemental groups (group B and E) but it was lower ($p<0.05$) than the control group A.

Table VI.- Sodium, potassium and chloride concentration in blood serum of birds fed experimental rations.

Treatment	Sodium (g/kg)	Potassium (g/kg)	Chloride (g/kg)
A (Control)	305.8[a]	30.0[a]	0.325[a]
B (Betaine)	229.4[b]	26.6[b]	0.325[a]
C (Vitamin C)	248.5[b]	25.9[b]	0.444[a]
D (Vitamin E)	238.9[b]	26.3[b]	0.390[a]
E ($NaHCO_3$)	229.4[b]	25.6[b]	0.346[a]

[a,b]Means within a column lacking common superscript differ significantly (P<0.05) separated by least significant difference test

Anti-NDV anti-body titers

Haemagglutination Inhibition (HI) titers were higher (p<0.05) when birds were fed on diets containing $NaHCO_3$ compared to all other experimental diets (Table V). It was also interesting to note that all birds fed feed nutrients showed comparatively higher (P>0.05) anti ND titers compared to unsupplemented control group.

Serum sodium, potassium and chloride concentration

The maximum serum Na and K concentrations were recorded in birds fed on control diet compared to all other supplemented diet groups (Table IV). There was no difference (P>0.05) in the serum Cl concentrations between all experimental groups.

Mortality

Highest (P<0.05) mortality (33%) was recorded in group A (control without any supplement) compared to all other supplemental groups (Table V) and. Among different supplemental groups the lowest (P<0.05) mortality was observed in birds fed diets containing betaine (3.3%) & $NaHCO_3$ (6.6%). It was interesting to note that mortality only occurred during the last week of the experiment when RH % was high (Table II) and as a result the study was terminated at day 35.

DISCUSSION

Growth performance

In the present study the body weights of chickens were lower than those expected at 35 days of age demonstrating the adverse effects of high ambient temperatures coupled with the high humidity experienced by the birds throughout the experimental period. The average high daily temperature and RH values reduced the feed intake and body weight gains. The poor performance of the birds, especially those fed the control diet was consistent with reports by others (Balnave and Oliva, 1991; Balnave and Gorman, 1993; Hayat *et al*., 1999). The present findings indicated that the role of supplements in poultry feed was prominent when RH values increased above 65% *i.e.*, in weeks 4 and 5. The birds in group E, where $NaHCO_3$ was added at 2 g/kg gave consistently better (P<0.05) performance in terms of weight gains and FCR as compared to all other supplemental groups. The use of supplemental $NaHCO_3$ in poultry feed is beneficial as it is a supplemental source of Na^+ as well as HCO_3^- ions to replace CO_2 which is lost to a greater extent during panting (Gorman and Balnave, 1994). The results presented here are in agreement with Gorman and Balnave (1994), who evaluated twelve different dietary mineral supplements in hot weather (30°C) and noted better body weight gains and FCR in birds fed $NaHCO_3$ as a supplement. Similarly Hayat *et al.* (1999) revealed that supplementation with $NaHCO_3$ (2g/l) caused increased growth and feed intake compared to un-supplemented controls in heat stressed environmental conditions.

In the present study highest feed intake (P<0.05) of birds fed VC supplemented at day 35 compared to birds fed the unsupplemented diets (Figure-2) is in agreement with the work of McKee and Harrison (1995) who reported higher feed intake in broilers supplemented with ascorbic acid as compared to the group without ascorbic acid in heat stress conditions. It has been reported that the ability of poultry to synthesize ascorbic acid is inadequate under stress conditions, such as high environmental temperatures, high humidity, a high productive rate and parasitic infestation (Pardue *et al.,* 1986; Pardue *et al.*, 1984). Thus supplementation of diet with VC can be beneficial due to its known metabolic functions as it serves as a classical enzyme cofactor, as a protective agent and as an ascorbyl radical in interactions with transition metal ions (Combs, 1992). The present study also supports the work of Blaha and Kroesna (1997) who demonstrated that ascorbic acid supplemented birds (ascorbic acid at 20 mg in drinking water) had significantly better FCR and higher weight gains compared to an unsupplemented group.betaine improved FCR of birds exposed to high environmental temperature (above the thermoneutral zone) compared to the birds without supplementation during heat stress (Ombabi, 2004; Simon, 1999; Virtanen *et al*., 1993). This positive effect of betaine may be related to the osmolyte function of betaine that reduces dehydration (Kettunen *et al*., 2002). Kettunen (2001) reported that in the presence of betaine in hyperosmotic medium the reflux of water into duodenal and jejunal slices was reduced, indicating that betaine has an effect on the movement of water across the intestinal mucosa. Evidence suggests that betaine is a valuable nutrient itself (Eissen and Enting, 2007) while Kidd *et al*. (1997) reported that betaine has two primary metabolic roles, as a methyl donor and as an osmolyte that assists in cellular water homeostasis. Belay *et al.* (1992) also reported better FCR with chickens fed diets containing betaine compared to an unsupplemented group.

Similarly in agreement with the present study, the importance of different feed supplements in improving overall growth performance was also acknowledged by Sahin *et al.* (2003) and Mezes (1999) who studied the effects of antioxidant vitamins in poultry nutrition and showed that VE as biological antioxidant, plays an important protective role against the negative effects of heat stress and results in better FCR compared to unsupplemented groups under harsh environmental conditions. Furthermore, Combs (1992) while explaining the metabolic role of VE reported that the antioxidant function of VE involves the reduction of free radicals thus protects the cells of the body against the potentially deleterious reactions of highly reactive oxidizing species.

Anti-NDV antibody titres

It was observed in the present study that heat stress reduces the immunity of birds against Newcastle disease virus vaccine. However highest ($P<0.05$) immunity was observed when birds were fed diets supplemented with $NaHCO_3$ during the period of heat stress. These results are in agreement with the work of Mashaly *et al*. (2004) who reported that birds under heat stress showed an increased heterophil/lymphocyte ratio and a decrease in antibody titres, thus it can be suggested that birds with $NaHCO_3$ in the diet were least effected by heat stress.

Mortality

The highest mortality in group A (control) compared to all others nutrient supplemented groups in the present study reflects similar trends observed in commercial enterprises where birds are kept in open sided houses under similarly hot and humid conditions (Abou-El-Ella and Ismail, 1999; Ahmad *et al.*, 2005). However supplementation of the diets caused a large decrease in mortality and is in agreement with work reported by Chew (1995). The results of mortality in this study showed that the tolerance against different stressors like temperature and humidity decreased as the heat stress period increased. These results are in agreement with the work done by Chew (1995) and Hayat *et al.* (1999) who showed lower mortality in birds supplemented with betaine, VC, VE and $NaHCO_3$ compared to un-supplemented control in heat stress conditions.

Blood pH

The highest blood pH value was found in group A compared to all other supplemented groups. These results are in agreement with the work reported by Teeter *et al.* 1985; Branton *et al.* (1986), and Belay and Teeter (1993) who showed that heat stress causes rapid shallow breathing inducing respiratory alkalosis thus increasing blood pH. Among the supplemented groups the lowest pH value was found in serum of birds fed VC (group E) and highest values were observed in birds fed $NaHCO_3$ (group E). These results were similar to the work of Keskin and Durgun (1997) who showed that supplementation of $NaHCO_3$ in diet significantly increases the pH in the blood from quail.

The blood pH should be close to physiological limits (pH; 7.35- 7.45) (Dibartda, 1992; Carlson, 1997). This is necessary for the maintenance of protein structure and function, which is an essential condition for normal progression of metabolic events. Normally, blood pH is controlled by the lungs and kidneys along with the various buffer systems which prevent rapid changes in pH. However, the immediate response to heat stress is that respiratory rate increases and a corresponding decrease in the levels of blood carbon dioxide and respiratory alkalosis (elevated blood pH) is observed (Borges *et al.*, 2003).Thus the elevated levels of blood pH in the present study clearly indicates that the birds were in heat stress.

Total leukocytic count

Generally, normal TLC in chickens (*Gallus gallus domesticus*) were 1.2-3.0 $\times 10^4$ cells/µl (Jain, 1993). A count that is greater than the normal range is considered suggestive of leukocytosis. General causes of leukocytosis include stress, oestrogen administration, disease and certain drugs (Ritchie *et al*., 1994; Aengwanich *et al*., 2003). Similarly, Reddy (2000) reported that when broilers were exposed to high ambient temperature, their body temperature increased, corticosterone stored in adrenal cortex was released into the blood circulation to help broilers increase their metabolism. This hormone might cause cell mediated and humoral immunity failure because changes in the plasma concentration of corticosteroids and ACTH affected the lymphoid tissue (Richard, 1998). Furthermore, Jain (1993) reported that corticosteroid in induced lymphopenia attributed to lympholysis in blood and lymphoid tissue, increased shift of lymphocytes from blood to other body compartments, or both. T-cells in blood and tissue are most sensitive to lympholytic effect. Lymphocytes have high affinity receptors for corticosteroids in their cytoplasm. After ligand receptor complexes bind to specific DNA sequences and induce the synthesis of mRNA, which in turn triggers the synthesis of protein that inhibits intracellular glucose transport and lipid synthesis then an endonuclease may become activated, causing DNA fragmentation. Glucocorticoids also markedly inhibit the synthesis of IL-1 by macrophages and IL-2 by activated T cell, thereby thwarting an immune response (an immunosuppressive effect). Similarly, Nockel (1973) reported that ascorbic acid could decrease corticosterone level in the circulation when broilers are exposed to high ambient temperature.

The TLC values in the present study were higher ($P<0.05$) than the normal range in the unsupplemented group (A) compared to supplemented groups. In agreement to the present study, Sahin and Kucuk (2001) found that dietary VE inclusions resulted in a greater performance in Japanese quails reared under heat stress 34°C. They further reported that dietary supplements can modify gene expression induced by heat shock *in vivo* and has a protective role against oxidative stress by enhancing the level of indigenous antioxidants and inducing hsp-70 gene expression (Ushakova *et al*., 1996). Furthermore, Sahin *et al*. (2002) found that heat stress tended to elevate plasma corticosterone concentrations which were significantly reduced with VE supplementation in a diet of Japanese quail reared under heat stress conditions (34°C). In addition increasing concentration of ACTH was parallel to increase in serum glucose, uric acid and triglycerides concentration. They suggested that this increase was due to a greater catabolic effect (or concentration) of ACTH. Thus it might be suggested that dietary supplements used in the present study reduced the heat stress either by decreasing the plasma corticosterone level or modified gene expression induced by the hsp-70 gene expression.

Serum sodium, potassium and chloride concentration

As a result of severe heat stress the birds started panting as a mean of reducing their temperature. Panting leads to disturbances in acid base balance of the bird (Arad *et al.,* 1982). Respiratory alkalosis is very evident from the data of blood pH of this experiment. Birds, fed on the control diet exhibited the most alkalinity whereas minimum alkalinity was noted in birds fed on diets containing sodium bicarbonate. Betaine, which is considered as substitute of bicarbonate (Teeter *et al*. 1985) and osmoregulator (Jones, 1975) and vitamin C (Pardue and Thoxton, 1986) has role in corticoid synthesis. Of the treated groups the highest pH was in the group fed on vitamin E. The role of vitamin E is not likely to regulate the acid base balance so the highest pH is reported in this group.

Birds fed on non supplemented control diet (group A) exhibited highest pH and highest levels of Na and K ions. The reason for the variation in serum minerals could the disturbances in acid-base balance of birds due to severe heat stress exposure (Ahmad *et al*., 2005). Birds were found to be under continuous panting during last three weeks of experimental period. Panting result in respiratory alkalosis as reported by names of authors and as found in this experiment. Respiratory alkalosis is characterized by excessive removal of blood carbon dioxide (Keskin and Durgan, 1997). Carbon dioxide in the blood is a source of H_2CO_3. This is a source H ion in the blood a lack of H ion can lead to poor Na and K reassertion, causing more Na and K in the urine and leaving less Na and K in the blood. (Orloff and Davidson, 1959).We can see the similar phenomenon under present experimental conditions. This was also reported by Kohne and Jones, 1975; Teeter *et al.*, 1985). The highest levels of Na were reported in control (non supplemented group). The reason for this phenomenon was that birds were under severe heat stress conditions. In last three weeks of experiment environmental temperature remained 8 to 12 °C higher than thermo neutral zone for that age group

Respiratory alkalosis also alters the anion/cation balance. This in turn has a significant effect on metabolism. A reduction in body weight gain in broilers is observed from experimentally induced ion imbalance. (Mongin, 1981), apart from the reduced growth during heat stress conditions can be attributed to cation imbalance. The mechanism of reduction in circulating mineral levels under heat stress has been explored by various researchers. Poor absorption of Ca, K, and P was indicated in turkeys (Wolfenson *et al.,* 1987) whereas, other reported reduced retention of Na, K, Ca, Cu, P and Fe in heat stressed chickens (El-Husseiny and Creger, 1981; Smith and Teeter, 1987; Belay *et al.,* 1992) reported increase excretion of these minerals through urine. Data of present study showed lower serum sodium on supplemented diets. A difference of 33 % sodium was reported in Bicarbonate and Betaine supplemented group. Similar trends were seen in case of K ions. In case of Cl there was no clear pattern of treatment results.

The lower serum K level in the supplemented groups compared to the control birds could be due to the fact that K ions shift between muscle and extra cellular fluid during heat stress to maintain cellular atmospheric pressure (Keskin and Durgan, 1997) or it may be due to reason that potassium excretion from kidney was increased and also the uptake was increased by erythrocyte and skin (Smith and Teeter, 1987). The results of present study are in agreement with the work done by Keskin and Durgan (1997) who reported that acute heat stress resulted in a sharp decline in blood CO_2 partial pressure accompanied by a fall in blood bicarbonate with the increase in pH in quail. Other researchers have also reported a reduction in plasma level of Na and K as a consequence of heat stress (Deyhim *et al*., 1990; Belay and Teeter, 1993; Ait-Boulahsen *et al.*, 1995; Borges, 1997). During acute heat stress as the body temperature rises haemodilution occurs, which causes a lowering of Na concentration and some of the tissue K exits into the blood stream apparently due to altered membrane permeability. When this K translocation phenomenon abates during or after acute heat stress and excess K is excreted (Smith and Teeter, 1987) plasma K concentration return to normal or below. However, regarding serum Cl values, no difference in Cl value was observed between serum and Cl which is not in agreement with the Ruiz-Lopoz and Austin (1993), who reported that due to heat stress and the resulting respiratory alkalosis, more Cl is needed in body fluids to exert an acidic action to normalize blood pH, thus blood Cl is increased after heat stress.

The results of serum sodium ion in group A (control) are different from all other supplemented groups. In four supplemented groups there is no difference in serum sodium concentration and in group A (control) serum sodium concentration was higher than all other four groups. It means the additional supplemented betaine, VC, VE and $NaHCO_3$ had almost similar effect on serum sodium concentration. In group A serum sodium level was high which may be due to more feed consumption by which these birds gained more sodium from the feed.

Botura (1993) reported an increase in blood K level in response to heat stress. This K response seems to be related to the time under stress because Borges (1997) subjected broilers to cyclic periods of stress for 6d and found a reduction in blood K level. Excess of circulating K competes for buffer anions from the renal tubular fluid, preventing the removal of some of H^+, which then has to be reabsorbed and may cause acidosis. Blood glucose concentration may increase as a direct response to a transit increase in the secretion of adrenaline, noradrenaline, and glucocorticoids (Kolb, 1984). When birds are exposed to high temperatures, they respond by increasing respiratory rate, which lead to a reduction in blood CO_2 levels. This reduction results in decrease in HCO_3 concentration due to the increase in HCO_3 excretion with a reduction of H+ excretion by the kidneys to maintain acid-base homeostasis and concentration of blood nutrients.

Serum chloride concentration had no significant difference (P>0.05). Their concentrations were similar in almost all groups whether supplemented or control.

CONCLUSIONS

It can be concluded that when ambient temperature and RH increases above 35°C and 65% respectively, negative effects on the broiler performance is observed in open sided houses. The results of the present study showed that dietary supplements improved the bird performance under heat stress conditions. However, each supplement functions differently and thus has a different mode of action. It is further suggested that $NaHCO_3$ (2g/kg) and betaine (1200mg/kg) improves immunity, reduce mortality under heat stress conditions and thus improves the health, welfare and productivity as well as the viability of poultry production where heat stress is a problem.

ACKNOWLEDGEMENTS

FMK is grateful to the financial support of the Commonwealth Fellowship Commission in the UK. SAC is financially supported by the Scottish Government.

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(Received 15 June 2009, revised 30 July 2009)