IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 5, Issue 1 (Sep.- Oct. 2013), PP 47-52
www.iosrjournals.org

Clinicopathological changes induced by heat stress, their resolution by minerals and vitamin C supplementation in quails

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Abstract: *The effects of water supplements i.e. sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl) and ascorbic acid were evaluated on feed intake, weight gain, Feed Conversion Ratio (FCR), serum potassium, serum bicarbonate level and on blood parameters in broiler Japanese quail (Coturnix coturnix japonica) exposed to high ambient temperature (34^0C, 8hr/d, 0900-1700hr). The quail chicks (n=300; 14 days old) kept at 34^0C (heat-stressed) were divided into five groups (B, C, D, E, F) of 60 chicks each. Group B with no water supplementations, group C supplemented with (250 mg of L-ascorbic acid/liter of water), group D (125mg of potassium chloride/liter of water), group E (75mg sodium bicarbonate/liter of water), group F (250 mg of L-ascorbic acid +75mg/liter sodium bicarbonate/liter of water, whereas group A chicks (n=60) kept at 22^0C were fed the basal diet (TN group). Heat-stressed quails of group B, lead to significant decrease ($P < 0.05$) in weight gain, serum potassium and serum bicarbonate level, erythrocytes, monocytes, lymphocyte proportions with increase in H/L ratio from 0.55 to 0.62 whereas the proportions of eosinophil and hematocrit values were not as much significantly affected. Groups (C, D, E and F) supplemented with Vit C, KCl and $NaHCO_3$ respectively at the level of 250 mg/L, 125gm/L and 75mg per liter of drinking water alone and in combination had significantly increased ($P<0.05$) serum potassium and serum bicarbonate level, erythrocytes, with improved weight gain and FCR. The results of the present study showed that supplementing a combination of vit. C and sodium bicarbonate in drinking water offered a good management practice to reduce heat stress-related decreases in broiler Japanese quail (Coturnix coturnix japonica).*

Keywords: *Heat stress, water supplements, vitamin C, sodium bicarbonate, potassium chloride, quail, H/L ratio*

I. Introduction

The stress of high ambient temperature may negatively influence the performance of broiler chickens by reducing feed intake, live weight gain and feed efficiency [1,2]. Broiler Japanese quail (*Coturnix coturnix japonica*) are farmed in large numbers, which are mostly kept to produce eggs that are sold worldwide and have tremendous potential for village and backyard production as well as an important laboratory animal [3]. This is because of their small size, the ease with which they can be kept, requiring only cages and equipments and little space [4], high eggs production reaching 300/year [5,6]. High ambient temperature is highly undesirable in broiler production. Its effect is always much greater when coupled with high humidity as water losses its ability to evaporate. Reduced feed intake, growth rate, feed conversion ratio and survivability are immediate consequences of rearing broilers in a hot humid environment [7]. Electrolytes are necessary to maintain function during hot weather [8]. Heat stress results in increased excretion of potassium through urine, hence results in decreased plasma potassium [9]. Heat exposed birds may exhibit reduced level of plasma carbon dioxide and bicarbonate [10]. Different therapeutic agents have been proposed as possible therapies to mitigate the negative effects of heat stress on the performance of broiler. These include ascorbic acid [11], vitamin B-complex [12], vitamin E [13], acetylsalicylic acid [14], sodium chloride [15], potassium carbonate, potassium chloride, ammonium chloride [16] and sodium bicarbonate [17]. Heat exposure reduces plasma carbon dioxide and bicarbonate [10], which may affect the blood pH and induce a nutritional requirement for bicarbonate [18]. Bonsembianate et al. [19] reported a level of 0.5% sodium bicarbonate stimulates feed and water intake in broilers. The main objective of this research was to investigate the effects of water supplements, ascorbic acid, KCl and $NaHCO_3$ separately and in combined form to heat-stressed broiler Japanese quails from day 14 to day 35, on their general body performances, weight gain, FCR, serum potassium, serum bicarbonate level and on hematology.

II. Materials And Methods

1.1: Animals, diets, experimental design and data collection. A total of three hundred and sixty, 14-d-old Japanese quails (*Coturnix coturnix japonica*; Avian Research and Training (ART) Centre, Millat Campus University of Veterinary and Animal Sciences, (UVAS) Lahore, Pakistan) were used in the study in August,

2010. The birds were assigned according to their initial same body weights, to six treatment groups, three replicates of sixty birds each. The birds were kept in cages (60 birds per cage) in temperature-controlled rooms, and the night time temperature for all groups was the same (22^0C). The birds kept at 34^0C for 8 h (0900-1700) were fed either a basal diet (high temperature, basal diet; HS group) or the basal diet supplemented with 250 mg of L-ascorbic acid/liter of drinking water (Vit. C group), 125mg of potassium chloride/liter of water (KCl group) and 75mg/liter of sodium bicarbonate/liter of water ($NaHCO_3$ group) or both (Vit. C + $NaHCO_3$ group), whereas Japanese quails (*Coturnix coturnix japonica*) kept at 22^0C were fed the basal diet (thermo neutral, basal diet; TN group). Feed intake and body weight were determined at weekly intervals, and over all weight gain and FCR of birds were calculated at 35^{th} day of age. Two millimeters of blood was collected using heparinized syringes after slaughtering four birds from each replicate. Whole blood was used for determination of packed cell volume and smear were prepared for H/L ratio, differential leucocytes count [20], Total leucocytes count [21], Heterophil lymphocytes ratio [22] and total erythrocytic count, blood sample for hematocrit were collected in heparinized capillary tubes and centrifuged in a micro hematocrit centrifuge for 7 minutes, for differential leucocyte counts two drops of blood were used and blood smear were made on duplicate glass slides. These smears were stained with wright stain in 15 minutes. One hundred leucocytes, including heterophils, lymphocytes, monocytes, basophils and eosinophils were counted on each slide. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes. Both slides were counted and the means were calculated for each bird. Another four millimeter of blood was collected on day 21, 22, 23, 24, 29, 30 and 31 in clean and dry vacutainer tubes; blood was allowed to clot, and the serum was used to measure serum potassium and bicarbonate level by spectrophotometeric method in the pathology department laboratory, University of Veterinary and Animal Sciences Lahore.

1.2: Statistical analysis: results are expressed as means ± SEM for each group. Groups were tested for differences by performing the one way ANOVA with the significant level of 0.05 and Completely Randomized Design (CRD) was applied.

Table 1. Layout of the experiment

Group	Treatment (Heat=34^0C, Vit. C=250mg/l, KCl=125mg/l, $NaHCO_3$=75mg/l of drinking water)	No. of Birds
A	Control (basal diet without any supplementation & heat stress)	60
B	Heat group (basal diet +heat+no vit or mineral supplemetation)	60
C	Heat + Vit. C	60
D	Heat + KCl	60
E	Heat + $NaHCO_3$	60
F	Heat + (Vit. C + $NaHCO_3$)	60

III. Results

Supplemental vitamin C, sodium bicarbonate and potassium chloride increased live weight and feed intake, and improved feed efficiency ($P< 0.05$) in heat stressed birds (Table 2). Performances and feed conversion ratio were also higher ($P< 0.05$) in treated groups compared with the HS group (Group B) and generally were greatest in group F (Table 2). Separately and in combination, supplemental vitamin C, potassium chloride and sodium bicarbonate increased serum concentration of K^+ and HCO_3^- ions ($P< 0.05$) (Table 3 and 4). Serum concentration of K^+ and HCO_3^- ions were greater ($P< 0.05$) in the TN group than in the HS group. Retention rates for K^+ and HCO_3^- ions were highest in the TN group (Table 3 and 4). Supplementing the diet with vitamin C, potassium chloride and sodium bicarbonate increased the retention of minerals, which was highest in group F and lowest in group B (HS) ($P< 0.05$) (Table 3 and 4). On the other hand, the excretion of minerals, which was lowest in group A, lower in treated groups than HS group ($P< 0.05$) (Table 4). Exposure of the Japanese quails to 34^0C significantly increased heterophil, basophil proportions and H/L ratios, and decreased erythrocytes, monocyte and lymphocyte proportions. Analysis of blood samples showed increase in total white blood cells ($P<0.05$), erythrocytes, lymphocytes, heterophils and eosinophils in heat stressed quails of group B (Table 5). While the basophils, packed cell volume and monocytes showed a significant decreased level in group B ($P<0.05$) while in group A and F, no significant difference ($P<0.05$) was observed. The heat distress caused a reduction in hematocrit and an increase in H/L ratio (Table 5), apparently associated with hemodilution, an adaptive response enabling water loss by evaporation without compromising plasma volume with most of the evaporative water loss coming from the extra cellular compartment. The results showed that increase ambient temperature (34^0C) was stressful in exposed group B and addition of minerals and vitamin C ameliorated the effect of high ambient temperature.

Table 2. Effects of supplemental vitamin C, potassium chloride and sodium bicarbonate on performance of Japanese quail reared under conditions of heat stress (34^0C) for day 14 to 35.

Item	Groups					
Live weight , g	TN(A)	HS(B)	Vit. C(C)	KCl(D)	$NaHCO_3$(E)	Vit. C+$NaHCO_3$(F)
Initial	101.5	101.1	101.9	101	102.4	101.7
Final	192	170	184.6	185.5	184	190
Total feed intake, g	533.76	533.8	531.65	534.24	531.76	532
Feed efficiency						
g gain/g feed	.36	.31	.35	.35	.35	.36
Feed Conversion Ratio	2.78	3.14	2.88	2.88	2.89	2.8

Table 3. Serum potassium level in quails exposed to heat stress 34^0C

	Days						
Groups	21	22	23	24	29	30	31
(Meq/L)							
A	5.16 ±0.025^a	5.15 ±0.018^b	5.13 ±0.019^c	5.1 ±0.018^e	5.11 ±0.013^g	5.11 ±0.022^i	5.1 ±0.01^k
B	5.1 ±0.025^a	4.9 ±0.012^b	4.76 ±0.017^d	4.73 ±0.017^f	4.5 ±0.015^h	4.49 ±0.019^j	4.45 ±0.01^l
C	5.09 ±0.025^a	5.08 ±0.001^b	5.11 ±0.017^c	5.16 ±0.001^e	5.2 ±0.01^g	5.21 ±0.021^i	5.19 ±0.026^k
D	5.07 ±0.022^a	4.99 ±0.011^b	4.88 ±0.021^d	4.8 ±0.008^f	4.62 ±0.022^h	4.55 ±0.022^j	4.48 ±0.020^l
E	5.08 ±0.022^a	4.97 ±0.011^b	4.91 ±0.012^c	4.81 ±0.008^e	4.71 ±0.007^g	4.64 ±0.011^i	4.61 ±0.006^k
F	5.12 ±0.022^a	5.03 ±0.017^b	5.06 ±0.017^c	5.12 ±0.022^e	5.1 ±0.009^g	5.09 ±0.016^i	5.1 ±0.008^k

Values with different superscripts in the column differ significantly ($P < 0.05$)
A= Control, B= Heat 34^0C, C= Heat 34^0C + Vit C (250 mg/l), D= Heat 34^0C + KCl (125mg/l), E= Heat 34^0C + $NaHCO_3$ (75mg/l), F= Heat 34^0C + (Vit C 250 mg/l+$NaHCO_3$ 75mg/l)

Table 4. Serum bicarbonate level in quails exposed to heat stress 34^0C

	Days						
Groups	21	22	23	24	29	30	31
(Meq/L)							
A	24.02 ±0.173^a	23.7 ± 0.071^b	24.02 ± .158^c	23.65 ± .158^e	22.86±.205^j	22.45 ±.162^k	22.59 ±.138^n
B	23.97 ±0.068^a	21.75 ±.132^b	18.48 ±.093^d	17.89 ±.098^g	13.39 ±0.039^j	13.03 ±0.046^l	12.9 ±0.031^0
C	22.96 ±0.079^a	20.03 ±.054^b	18.44 ±0.10^d	15.57 ±0.07^g	13.11 ±0.04^j	12.83 ±0.015^l	12.74 ±.037^0
D	25±0.134^a	24.06 ±.068^b	23.47 ±.048^c	20.38 0.072^f	17.58 ±0.081^i	17.07 ±.058^m	16.56 ±.068^p
E	22.39 ±0.311^a	21.07 ±.098^b	18.5 ±0.103^c	15.8 ±0.085^g	13.19 ±0.112^i	13.09 ±.064^m	12.54 ±.073^p
F	24.5 ±0.118^a	24.1±.141^b	23.6±.068^c	21.9±.072^f	18.65±.068^i	17.99±.065^m	21.99±.051^n

Values with different superscripts in the column differ significantly ($P < 0.05$)
A= Control, B= Heat 34^0C, C= Heat 34^0C + Vit C (250 mg/l), D= Heat 34^0C + KCl (125mg/l), E= Heat 34^0C + $NaHCO_3$ (75mg/l), F= Heat 34^0C + (Vit C 250 mg/l+$NaHCO_3$ 75mg/l)

Table 5. Heamatological parameters in quails exposed to heat stress 34^0C

Parameters	Groups					
	A	B	C	D	E	F
RBC($\times 10^6$/mm^3)	3.95 ±0.009	3.04 ±0.043	3.51 ±0.043	3.54 ±.008	3.53 ±0.018	3.59 ±0.053
WBC ($\times 10^3$/mm^3)	3.8 ±0.048	4.24 ±0.041	4.24 ±0.041	4.01 ±.012	3.99 ±0.047	3.96 ±0.037
Lymphocytes (%)	56.1 ±0.045	54.19 ±.098	57.05 ±0.02	57.01±.012	56.93 ±0.014	56.77 ±0.12
Heterophils (%)	30.9 ±0.039	33.6 ±0.129	33.09 ±0.01	33.06±.035	33.02 ±0.09	32.93 ±0.09
H/L Ratio	0.55 ±0.03	0.62 ±0.02	0.58 ±0.03	0.58 ±0.01	0.58 ±0.03	0.58 ±0.02
Basophils (%)	1.5 ±0.015	1.63 ±0.013	1.62 ±0.015	1.6 ±0.017	1.61 ±0.013	1.6 ±0.01
Eosinophils (%)	2.19 ±0.017	2.21 ±0.02	2.19 ±0.008	2.15 ±.015	2.16 ±0.034	2.13 ±0.035
Monocytes (%)	6.3 ±0.036	5.59 ±0.025	5.42 ±0.011	5.43 ±0.11	5.89 ±0.007	5.92 ±0.064
PCV (%)	40.5±0.117	37.19±0.1	38.02±0.088	38±0.562	37.93±0.135	39.25±0.09

A= Control, B= Heat 34^0C, C= Heat 34^0C + Vit C (250 mg/l), D= Heat 34^0C + KCl (125mg/l), E= Heat 34^0C + $NaHCO_3$ (75mg/l), F= Heat 34^0C + (Vit C 250 mg/l+ $NaHCO_3$ 75mg/l)

IV. Discussion

Significant negative effects on live weight gain, feed intake, feed efficiency, mineral and hematological status occurred in quails exposed to high ambient temperature (34^0C). In the present study, vitamin C and sodium bicarbonate supplementation in combination increased feed intake, improved growth rate, blood parameters, retained potassium and bicarbonate ions, as same results were documented by [2, 23]. High environmental temperatures are known to decrease weight gains in chicks as observed in this study which could be due to several factors including decreased feed consumption, decreased digestion [24] and impaired metabolism [25]. Suk and Washburn [26] have shown decreased efficiency of feed utilization with increased environmental temperatures and are of the opinion that decreased feed consumption is closely related to extra heat load accumulated in the course of heat stress. A few earlier studies have reported that alternate management protocols and dietary modifications can alleviate the adverse effects of chronic heat-stress in broiler Japanese quails (*Coturnix coturnix japonica*) chicks [9]. Correction of blood acid-base imbalance may be achieved by electrolyte supplementation through drinking water or feed. Naseem et al. [27] documented that $NaHCO_3$ and KCl have ameliorated heat-stress induced disturbances in acid base balances.

Vitamin C, potassium chloride and sodium bicarbonate similarly affected all variables measured in the present study. In addition, for most variables, the magnitude of the effect was greater when both were supplemented compared to either compound given alone. Al- though ascorbic acid does not appear to be needed for normal folate metabolism, lower ascorbic acid concentrations occur in folate deficiency and utilization of folic acid is impaired in ascorbate deficiency, suggesting an interaction between the vitamins [28, 29]. In addition, antioxidant activity was re- ported to be more efficient when antioxidants are used in combination [30].

The present study resulted improved FCR, lower H/L ration, increased RBCs level and improved hematocrit values in groups other than heat-stressed group which was due to potassium chloride, sodium bicarbonate and vitamin C supplementation respectively completely agreed with the earlier research findings of Lopez and Austic [31] who reported improvement in FCR after electrolytes supplementation [9]. Environmental stress increases mineral excretion [32]. Similar results were obtained in the present study, El-Husseiny and Creger [33] reported significant lower rates of retention of minerals such as potassium and sodium in broilers subjected to environmental stress. The improvement in weight gain in $NaHCO_3$ supplemented chicks observed in this study may be due to the fulfillment of HCO_3^- requirement, otherwise decreased due to respiratory alkalosis induced by heat stress [18]. Serum potassium levels decrease during heat stress which may be due to extracellular fluid, increased renal excretion, increased potassium uptake by erythrocytes and/ or skin [34, 35], and a reduced competition between H^+ and K^+ ions for urinary excretion resulting in increased urinary potassium loss [36]. Heat-stressed quail birds given 125mg/l potassium chloride solution exhibited significantly higher serum potassium levels than non supplemented groups which are in line with the results reported by [27]. Weight gains and improved FCR observed in quail chicks supplemented with combination of vitamin C and $NaHCO_3$ in this study are similar to the findings of the researchers [10, 27, 9, and 31]

Heat stress caused changes in the proportions of circulating erythrocytes and leucocyte components. Exposure of broiler Japanese quails (*Coturnix coturnix japonica*) to high temperature exhibited a significant decrease in lymphocytes and raised heterophil ratio. In group B, the H/L ratio increased from 0.55 to 0.62. Heat-stressed Japanese quails responded with a significant increase in basophils. These findings are in agreement with the findings of Mitchell et al. [37] who observed an increase in basophil counts after heat stress. Maxwell et al. [39] suggested that an increase in the H/L ratio may be a response to mild or moderate stress but a basophilia may result from extreme stress [40]. Although the H/L ratio of 0.62 showed optimum stress, according to Gross et al. [22], basophilia observed in this study showed that exposure of broiler Japanese quail (*Coturnix coturnix japonica*) at 34°C for 8 hours/ day for 18 days produced significant negative effects in these quail chicks. Exposure of the quail birds to acute heat stress resulted in a decreased monocyte proportion whereas the eosinophil proportion was not affected (Table 5).

in this study heat stressed Japanese quails (*Coturnix coturnix japonica*) supplemented with vitamin C, potassium chloride and serum bicarbonate alone and in combination showed only a small decrease in erythrocytes, packed cell volume, eosinophil and monocyte numbers, the present study results are in partial agreement with the research findings of Maxwell et al. [39] who stated that broilers given limited feed showed only a small decrease in eosinophil and monocyte numbers. In the review of Maxwell Robertson [41], it was reported that eosinophils disappear from circulation and basophils increase in circulation during stress, particularly in acute stress. Yahav, S and S. Hurwitz [42] reported decreased pack cell volume due to exposure to heat stress 34^0C. These findings are in agreement with the research findings of Zhou et al. [44] the present study in which heat-stressed group not supplemented with vitamins and mineral showed decrease in pack cell volume as reported by. While Altan et al. [45] did not notice any change in PCV following exposure to heat 39^0C for 2 h at 44 day of age, which are in contradiction to the results of the present study that might be due to short duration of heat exposure.

V. Conclusion

It is concluded that dietary vitamin C, potassium chloride and sodium bicarbonate have a synergistic effects, and as evidenced from the performance, serological and hematological results, supplementing a combination of dietary vitamin C and sodium bicarbonate offers a good management practice to reduce heat stress-related decreases in broiler Japanese quails (*Coturnix coturnix japonica*).

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