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Comparative Efficacy of Different Supplement Used to Reduce Heat Stress and Their Impact on Performance, Immunity and Some Biochemical Parameters in Broilers Chickens

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EFFECTS of fed supplements, sodium bicarbonate ($NaHCO_3$), Ascorbic acid (Vitamin C) and Vitamin E were evaluated on the performance, immune system and biochemical parameters of chronically heat-stressed broiler chicks. Two hundred day old broiler chicks were allotted to one of the five groups (n = 40),(A) normal control [no supplements], (B) heat stressed [no supplements], (C) $NaHC0_3$,2g/kg diet, (D) Vitamin C, 200mg/kg diet and (E) Vitamin E, 300 mg/kg diet. All chicks except group (A) were maintained at 35-40 ºC from day one to 6 weeks (wks) of age (daily 6 hrs heat stress episodes). Samples were taken at 2,4 and 6 weeks of age. The result showed Significantly higher bodyweight gains were recorded in all supplemented groups as compared to control group with better response in birds fed sodium bicarbonate ($NaHCO_3$) .Bursal index, percentage weights of thymus and spleen in relation to body weight, an indicator of humoral immunity were higher but the heterophil / lymphocyte ratio, an indicator of stress was lower for the all supplemented groups as compared to control group .The dietary supplement significantly increased antibody titer of ND and AI on day12 post vaccination ($P<0.05$). However, dietary vitamin E had higher effect on antibodies titer compared to other groups. In heat stressed group serum uric acid, urea, glucose, AST, ALT increased significantly ($P \leq 0.05$) while blood Na and K and total proteins, albumin, globulin, decreased significantly ($P<0.05$) from respective control mean values.There were no significant ($P>0.05$) effects of supplemental sodium bicarbonate ($NaHCO_3$), Ascorbic acid (Vitamin C) and Vitamin E on plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Overall, the results indicated significant improvement in the performance and immune response of chronically heat-stressed broiler chicks given the feed supplements, ascorbic acid supplementation enhanced the hematological profile of birds but generally, $NaHCO_3$ being slightly superior to Vitamin C and Vitamin E.

Keywords: Vitamin C, Vitamin E, Supplements, Bursal index.

Poultry production in tropical and semitropical countries is affected by many challenges especially during the hot humid summer season. Economic losses result from decreased productivity and increased mortality due to acute heat stress (Ahmed and Gopal, 2012). Broiler chickens are more sensitive to high

ambient temperaturecompared to other species of domestic animals. They have no sweat glands, a rapid metabolism, and high body temperature. Furthermore, fast-growing meat broilers generate more heat than their free-living counterparts living in the wild (Geraert *et al.,* 1996) and (Abidin & Khatoon, 2013).

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).

The heat stress causes an enhancement of core temperature and acceleration of breathing frequency. These changes lead to polypnea and a higher alveolar ventilation rate that is connected with evaporative cooling. The increased evaporation results in changes in blood gas and water concentrations in the internal environment of the organism. The tension of carbon dioxide decreases and the oxygen concentration increases in the blood. The respiratory alkalosis rectified by HCO_3 developed in the organism. It is due to the reduced reabsorption from the glomerular filtrate in kidneys (Hartlova *et al.,* 2002).High ambient temperature is very disruptive for broiler chickens and reduces feed intake, weight gain, carcass weights, and abdominal fat and increases mortality (Belay and Teeter, 1996).

The blood and circulatory system is particularly sensitive to changes in temperature, being an important indicator of physiological responses in birds to stressing agents. Borges *et al.* (2007) surmised that quantitative and morphological changes in blood cells were associated with heat stress and were translated by variations in haematocrit value, leukocyte counts, erythrocytes and hemoglobin contents.

Several methods have been proposed for reducing heat stress effects in poultry including nutritional methods. 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 or to supplement the diets with different additives such as vitamin C (Whitehead and Keller, 2003), vitamin E (Ajakaiye *et al.,* 2010), sodium bicarbonate (Hayat *et al.,* 1999). A number of studies have shown beneficial responses to different feed additives independently however, little studies have been conducted to directly compare the effects of different feed supplements at the recommended concentrations.

So, this study was planned to compare effect of different supplements used in field to ameliorate the negative effect of heat stress on chicken from different aspects, body performance, immunity, biochemical parameters.

Material and Methods

Animals and experimental design

A total of two hundred, day old broiler chicks (Hubbard) were purchased from Ismailia/ Miser Poultry Company. The birds were reared throughout the entire period of study in the well ventilated, well adapted room during the experimental period. Then the birds were randomly divided into five equal groups, each consisting of 40 and numbered them as group A, B, C, D and E.

- Group (A) was maintained as thermo-natural control and was fed only with commercial broiler ration throughout the experimental period in normal temperature schedule.
- Group (B) was maintained as heat stressed and was fed only with commercial broiler ration throughout the experimental period.
- Group (C) was fed on commercial broiler ration supplemented with 2 g sod. Bicarbonate ($NaHCO_3$) /kg of diet.
- Group (D) was fed on commercial broiler ration supplemented with 200 mg of (Vitamin C) L-ascorbic acid/kg of diet.
- Group (E) was fed on commercial broiler ration supplemented with 300 mg vitamin E/kg of diet. Vaccination schedule was maintained properly for ND and AI

All birds except group (A) were exposed to 35- 40ºC/6 hrs. from 010.00 am to-04.00 pm (6 hrs heat stress episodes) daily during the experimental period by using the electric heater 2000 watts placed at 50 cm height of floor and heat distributed by fan.

Samples collection

At the 14^{th}, the 28^{th} and the 42^{th} day of exposure, individual blood samples collected from 10 birds (wing vein) in plastic tubeswith anticoagulant (EDTA) for hematological studies and other without anticoagulant, centrifuged at 3000 rpm/15min. to separate sru which kept in -18°C until biochemical analysis.

Measurement of live body weight parameters and mortality %

Live weight of 10 birds were randomly selected within replicates of each group and measurements were recorded with a standard electronic weighing balance At 2,4 and 6 weeks of age and mortality % were recoded.

Immunological studies

Weight of lymphoid organs: Six birds were randomly selected from each group, slaughtered and lymphoid organs (thymus, spleen and Bursa of Fabricius) were dissected out, weighed and calculated according to the formula of (Halouzka, 1991).

Haemagglutination inhibition (HI)test

It was performed using avian influenza and Newcastle disease virus antigens to determine antibody titers against avain influenza and Newcastle disease virus according to OIE (2012).

Hematological analysis

Blood smears were made and stained with Giemsa stain for differential leukocytes count recording heterophil H%, L% and heterophil/ lymphocyte (H/L ratio) (Gross and Siegel, 1983). RBC counts were done with the help of a hematocytometer by using the technique by Natt and Herick (1952), Hemoglobin concentration was determined by Sahli`s Acid Hematin method, PCV was obtained by the microhematocrict method, Hemocytometer was used forTLC and the procedure of Sastry (1989) was adopted. Blood pH value was estimated according to (Peter and Takeo,1975)

Biochemical analysis

Serum total protein, albumin and globulin were estimated by modified Biuret and Dumas method (Varley *et al.,* 1980). AST, ALT (Reitman's and Frankel, 1957), glucose, urea, uric acid were determined using kit from (BioMeriux – France) by Auto analyzer Hitachi 912.Serum sodium and potassium were estimated (Bold *et al.,* 1965).

Statistical analysis

All data were analyzed using the One-Way ANOVA procedure for analysis of variance. Significant differences among treatments were identified at 5% level by Duncan's multiple range tests (Duncan, 1955).

Results

Live body weight and mortality%

The result was illustrated in Table 1. The overall body weights were lower in heat stressed group than control group clearlyindicatednegative effectsofheat stress which also affected on mortality %.The birds fed on diets containing the dietary supplements gave variable degrees of improvements in weight gains (P<0.05) and mortality %.However, supplemented diet with $NaHCO_3$ showed better body weight gain followed by Vitamin C feed group which consequently reflected on mortality%. Heat stressed group have high morality % 22.5 than other groups.

Blood pH

Table 2 showed the effect of different feed supplements on blood pH of heat-stressed chicks.Heat stress elevates blood pH which regain around normal vales by using feed supplements especially $NaHOC_3$.

Hematology

Effect of different feed supplements on hematological parameters of heat-stressed chicks was presented in Table 3.

TABLE 1. Effect of different feed supplements on body weights (grams) and mortality % of heat-stressed chicks .

Age in weeks	Control (thermonatural) Group (A)	Heat stressed Group(B)	$NaHCO_3$ Group (C)	Vit C Group (D)	Vit E Group (E)
2	390.7±2.1[a]	255.7±6.9[d]	380.9±2.4[a]	355.1±4.9[b]	350.7±5.2[b]
4	1013.0±4.3[a]	830.0±2.9[d]	995.0±5.1[a]	945.4±5.1[b]	900.0±2.3[c]
6	1980.3±5.3[a]	1601.7±27.4[d]	1950.3±5.3[a]	1868.6±6.7[b]	1794.3±5.7[c]
Mortality(%)	0/40 (0)	9/40 (22.5)	1/40 (2.5)	2/40 (5)	2/40 (5)

*Treatment means within a week with different superscript letters are significantly different $p<0.05$.

TABLE 2. Blood pH of different feed supplements groups .

Age in weeks	Control (thermonatural) Group (A)	Heat stressed Group (B)	$NaHCO_3$ Group (C)	Vit C Group (D)	Vit E Group (E)
2	7.37±0.02[a]	*8.35 ±0.08[c]	8.01 ±0.03[b]	8.11±0.04[c]	8.21±0.07[c]
4	7.39 ±0.04[a]	*8.40 ±0.02[c]	8.02±0.06[b]	8.13±0.05[c]	8.23±0.02[c]
6	7.40 ±0.02[a]	*8.42 ±0.11[c]	8.04±0.11[b]	8.14±0.02[c]	8.24±0.10[c]
Mean	7.38 ±0.12[a]	*8.39 ± 0.03[c]	8.02 ±0.01[b]	8.12 ±0.01[c]	8.22 ±0.01[c]

*Treatment means within a week with different superscript letters are significantly different $P<0.05$.

TABLE 3. Effect of different feed supplements on some hematological parameters of heat stressed chick .

Parameters in weeks	Treatment				
	Control (A)	Heat stressed(B)	$NaHCO_3$ (C)	Vit.C (D)	Vit.E (E)
Red blood cells ($\times 10^6/\mu l$)					
2	2.90±0.22[a]	3.25±0.28[b]	2.14±0.19[c]	2.95±0.21[a]	2.50 ±0.19[c]
4	3.04±0.25[a]	3.79±0.29[b]	2.48±0.20[c]	2.72±0.26[a]	2.11 ±0.23[d]
6	2.54±0.28[c]	3.61±0.22[b]	2.21±0.15[c]	2.88±0.29[a]	2.51 ±0.21[c]
Mean	2.82 ±0.25[a]	3.55± 0.27[b]	2.27 ±0.18[c]	2.85 ±0.27[a]	2.37 ±0.22[c]
Haemoglobin (g/dl)					
2	10.2 ±0.2[a]	9.4±0.15[b]	9.66±0.18[b]	9.91±0.15[ab]	9.95 ±0.11[ab]
4	10.2 ±0.13[a]	9.2 ±0.14[b]	10.1±0.20[a]	10.30±0.18[a]	8.95 ±0.14[b]
6	10.3 ±0.14[a]	9.6 ±0.16[b]	9.54±0.09[b]	11.00±0.12[c]	10.1 ±0.22[a]
Mean	10.23 ±0.16[a]	9.4 ±0.14[b]	9.76±0.15[b]	10.40±0.15[a]	9.66 ±0.17[ab]
Packed cell volume (PCV)%					
2	30.6 ±0.35[a]	28.2 ±0.22[b]	28.98 ±0.15 [b]	29.77±0.16[ab]	29.10±0.14[a]
4	30.6 ±0.25[a]	27.6 ±0.25[b]	30.30 ±0.32[ab]	30.90±0.12[a]	30.7±0.17[a]
6	30.9 ±0.31[a]	28.8 ±0.27[b]	28.62 ±0.22b	33.00±0.24[c]	31.50±0.11[ac]
Mean	30.7 ±0.30[a]	28.2 ±0.25[b]	29.30 ±0.32[ab]	31.21±0.14[a]	29.60±0.14[ab]

*Treatment means within a week with different superscript letters are significantly different $P<0.05$

Heterophil to lymphocyte ratio H/L

Heat-stressed group showed significantly higher H:L ratio at 2, 4 and 6 weeks of age compared to all supplemented groups (Table 4).

TABLE 4. Leukocyte profile and H/L ratios of chickens with different supplemented groups.

Indicators in weeks	Treatment				
	Control (A)	Heat stressed Control (B)	$NaHCO_3$ (C)	Vit.C (D)	Vit.E (E)
Total leukocyte counts ($\times 10^9$/l)					
2	3.54± 0.12^a	3.21 ± 0.14^b	3.49 ± 0.19^a	3.50 ± 0.16^a	3.56± 0.11^a
4	3.61 ± 0.13^a	3.01± 0.15^b	3.52 ± 0.17^a	3.51 ± 0.12^a	3.49± 0.14^a
6	3.66 ± 0.15^a	2.48 ± 0.11	3.53± 0.12^a	3.52 ± 0.17^a	3.50± 0.17^a
Mean	3.60 ± 0.13^a	2.90 ± 0.10^b	3.51 ± 0.16^a	3.51± 0.15^a	3.52± 0.13 ^{a}
Lymphocytes (%)					
2	58.51± 0.22^a	51.51± 0.24^b	58.73± 0.23^a	57.81± 0.26^a	58.24± 0.20^a
4	54.02± 0.25^a	49.20± 0.22^b	58.01± 0.22^a	57.40± 0.25^a	57.60± 0.22^a
6	49.20± 0.26^a	45.35± 0.21^b	57.80± 0.18^{ac}	56.90± 0.27	55.70± 0.21^c
Mean	53.90± 0.24^a	48.68± 0.23^b	58.17± 0.21 ^{a}	57.37^a± 0.24 ^{a}	57.15± 0.21^b
Heterophils (%)					
2	28.93± 0.17a	31.5± 0.14c	28.61± 0.18a	29.00± 0.11a	28.24± 0.10a
4	31.41± 0.14a	35.7± 0.12c	28.82± 0.10b	29.21± 0.14b	29.00± 0.12b
6	33.60± 0.12a	37.3± 0.19c	29.40± 0.111	29.20± 0.17b	29.90± 0.17b
Mean	31.28± 0.13a	34.8± 0.15c	28.93± 0.13b	29.13± 0.15b	29.03± 0.13b
Eosinophils (%)					
2	6.00± 0.04a	5.20± 0.02ac	6.53± 0.04a a	5.92± 0.02a a	6.21± 0.01a a
4	5.64± 0.01a	2.75± 0.01ac	5.83± 0.04a a	6.00± 0.01a a	5.82± 0.05a a
6	5.00± 0.01a	4.10± 0.02ac	6.20± 0.04a b	7.60± 0.03a a	6.40± 0.04a b
Mean	5.54± 0.02a	4.01± 0.01ac	6.17± 0.04aa	6.50± 0.02a b	6.13± 0.04aa
Basophils (%)					
2	3.63± 0.01a	3.83± 0.02a	3.44± 0.01a	3.81± 0.05a	3.43± 0.03a
4	3.80± 0.02a	3.95± 0.01b	3.04± 0.02a	3.21± 0.02a	3.23± 0.01a
6	3.80± 0.04a	4.1± 0.02c	3.00± 0.05b	3.20± 0.03b	3.20± 0.02b
Mean	3.72± 0.02a	3.96± 0.02c	3.13± 0.03b	3.38± 0.03b	3.25± 0.02b
Monocytes (%)					
2	3.14± 0.01b	3.16± 0.03	2.83± 0.02b	3.62± 0.01ab	4.14± 0.04a
4	7.31± 0.02a	7.40± 0.04	4.40± 0.02b	4.22± 0.02b	4.41± 0.05b
6	8.40± 0.01a	8.60± 0.05	3.60± 0.04c	3.10± 0.04c	4.90± 0.03b
Mean	6.23± 0.01a	6.38± 0.04	3.60± 0.03 c	3.62± 0.03c	4.43± 0.04b
H/L (Heterophils/ Lymphocytes ratio)					
2	0.49	0.61	0.49	0.50	0.48
4	0.58	0.72	0.50	0.51	0.50
6	0.68	0.82	0.51	0.51	0.57
Mean	0.59	0.71	0.50	0.51	0.52

Means values with different superscripts alphabets along the same row are significantly different. Results are expressed as means ± standard deviations H/L= Relation heterophil/lymphocyte .

Anti-body titers against Newcastle disease, avian influenza and infectious bursal disease

The results revealed heat stress significantly effect on antibody titers (Table 5). The dietary supplement significantly increased antibody titer of Newcastle (N.D) and avian influenza (A.I) on day 12 post vaccination ($p<0.05$). However, dietary vitamin (E) had higher effect on antibodies titer compared with other groups.

Lymphoid organs index

Heat stress decreased the ratio of the weight of bursa, spleen and thymus to body weight of birds. At 2, 4 and 6 weeks of age, birds receiving supplements showed higher bursa indexas well as increased percentages of thymus and spleen weight ratios as compared to heat-stressed group (Table 6).

Biochemical studies

Tables 7 and 8 showed Effect of different feed supplements on biochemical parameters and liver and kidney function of heat-stressed chicks.

TABLE 5. Effect of different feed supplements on antibodies mean titer against Newcastle and avian influenza diseases 12 days post vaccination in heat stressed chicken.

	Control group (A)	**Heat stressed group (B)**	**NaHCO3 group (C)**	**Vit C group (D)**	**Vit E group (E)**
ND Newcastle	4.12	2.21	3.30	3.64	3.95
AI avian influenza	3.85	2.42	2.95	3.05	3.55

TABLE 6. Effect of different feed supplements on bursa index and lymphoid organ body weight ratios of heat-stressed chicks.

Lymphoid organ ratio	**Age in weeks**	**Control (A)**	**Heat stressed (B)**	**$NaHCO_3$ (C)**	**Vit C group (D)**	**Vit E Group (E)**
Bursa index	2	0.36±.03a	0.16±.01c	0. 33±.02b	0.35±.13a	0.35±.03a
	4	0.47±.13a	0.33±.03d	0.36±.02c	0.40±.03b	0.45±.13a
	6	0.27±.10a	0.20±.09b	0.25±.02a	0.25±.09a	0.25±.10a
Thymus (%)	2	1.13±.10a	0.60±.12c	1.05±.03b	1.15±.12a	1.12±.15a
	4	0.60±.22a	0.31±.03d	0.40±.02c	0.49±.03b	0.59±.24a
	6	0.48±.11a	0.21±.03d	05.±0.35c	0.40±.03b	0.46±.12a
Spleen (%)	2	0.39±.13a	0.13±.01c	0.34±.03b	0.35±.01b	0.38±.15a
	4	0.26±.13a	0.15±.03c	0.18±.33b	0.25±.03a	0.25±.03a
	6	0.27±.02a	0.20±.03d	0.22±.12c	0.24±.03b	0.26±.03a

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$).

TABLE 7. Effect of different feed supplements on some biochemical parameters of heat stressed chicks .

Indicators in weeks	Treatment				
	Control (A)	**Heat stressed (B)**	**$NaHCO_3$ (C)**	**Vit.C (D)**	**Vit.E (E)**
Glucose (g/dl)					
2	143± 5.6a	175± 7.72b	145±4.83a	155±5.22	165±8.45
4	157±8.02a	180± 6.34b	150±4.38c	160±4.39	146±8.93
6	160±6.22a	187±5.29b	142±4.01c	151±6.04	149±9.22
Mean	153.33±9.07a	180±6.2b	145.6 ±4.04c	155.33±4.5	153.33±10.20
Total protein (g/dl)					
2	4.36±.62a	3.35±.22b	4.22±.02a	3.95±.02b	4.10±.02a
4	4.19±.55a	3.51±.42b	4.45±.02a	4.33±.02a	4.45±.02a
6	4.67±.30a	3.79±.35b	4.78±.02a	4.12±.02a	4.23±.02a
Mean	4.39±.42a	3.55 ± 0.2b	4.48 ±0.28a	4.13 ±0.19a	4.27 ± 0.17a
Albumin (g/dl)					
2	2.49±0.21	2.36±0.13	2.53±0.22	2.25±0.16	2.36±0.16
4	2.42±0.19	2.39±0.14	2.65±0.14	2.45±0.18	2.5±0.12
6	2.48±0.18	2.64±0.17	2.57±0.17	2.41±0.20	2.35±0.14
Mean	2.46±0.18	3.55±0.15	2.57±0.17	2.37±0.16	2.40±0.11
Globulin (g/dl)					
2	1.87±0.02	0.99±0.04	1.69±0.04	1.7±0.01	1.74±0.2
4	1.77±0.03	1.12±0.03	1.80±0.03	1.88±0.02	1.95±0.3
6	2.19±0.10	1.15±0.02	2.21±0.02	1.71±0.04	1.88±0.2
Mean	1.94±0.05	1.08±0.05	1.9±0.03	1.76±0.2	1.84±0.2
Potassium (mmol/L)					
2	5.80±0.5a	4.58±0.3b	5.82±0.1a	5.53±0.3a	5.34±0.3a
4	6.03±0.7b	4.98±0.6b	5.90±0.5a	5.58±0.5a	5.87±0.2a
6	5.58±0.9a	3.88±0.4c	5.74±0.3a	5.48±0.6a	5.51±0.2a
Mean	5.82±0.8a	4.48±0.5b	5.82±0.2a	5.53±0.54a	5.57±0.4a
Sodium (mmol/L)					
2	144.3±6.2a	122.2±4.27b	143.5±4.56a	146.2±5.32a	146.2±6.11a
4	145.5±5.22a	128.3±6.38b	146.2±5.39a	145.3±4.05a	141.3±4.33a
6	143.5±4.76a	123.5±5.73b	144.5±8.02a	143.5±5.71a	144.7±4.92a
Mean	144.34±6.39a	124.26±5.39b	144.7±6.04a	145±5.03a	144.06±5.93a

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$)

TABLE 8. Effect of different feed supplements on liver and kidney functions of heat stressed chicks.

Indicators in weeks	Treatment				
	Control (A)	Heat stressed (B)	$NaHCO_3$ (C)	Vit.C (D)	Vit.E (E)
Aspartate amino transferase, (IU/ l)					
2	238±7.9a	354±9.87b	237±5.2a	243±8.7a	239±8.6a
4	237±7.2a	355 ±7.4b	239±7.2a	245±7.9a	236±5.2a
6	235±6.45a	358 ±5.3b	240±3.4a	241±6.5a	243±8.3a
Mean	236.66±1.5a	355.66±2.1b	238.66 ±1.2a	243 ±2.1a	239.33 ±3.5a
Alanine amino transferase, (IU/l)					
2	72.4±2.4 a	79.65±1.81b	75.6±1.95a	76.2±2.19a	73.91±1.08
4	73.2±2.5a	79.91±2.1b	76.7±2.4a	74.1±1.87a	75.7±1.3a
6	76.12±2.31a	80.14±2.2b	74.1±2.4a	75.8±1.37a	75.8±2.1a
Mean	73.90 ±1.95a	79.90 ±2.24b	75.46±1.30a	75.36±1.11a	75.13±1.06a
Uric acid (mmol/l)					
2	0.29 ± 0.02a	0.38 ± 0.01b	0.29± 0.04a	0.30± 0.02a	0.30± 0.02a
4	0.28 ± 0.04a	0.40± 0.03b	0.27± 0.03a	0.32± 0.01a	0.32± 0.01a
6	0.30± 0.02a	0.41± 0.01b	0.29± 0.02a	0.29± 0.03a	0.33± 0.03a
Mean	0.29 ±0.03a	0.39± 0.02b	0.28 ± 0.4a	0.30 ±0.2a	0.31 ±0.2a
Urea (mmol/l)					
2	0.59± 0.02a	0.86± 0.025b	0.60± 0.01a	0.61± 0.01a	0.60± 0.03a
4	0.60± 0.01a	0.88± 0.021b	0.62± 0.01a	0.65± 0.01a	0.62± 0.01a
6	0.62± 0.03a	0.90± 0.026b	0.64± 0.04a	0.60± 0.05a	0.64± 0.02a
Mean	0.603 ±0.2 a	0.88±0.22 b	0.62 ±0.2 a	0.62 ±0.21 a	0.62 ±0.2 a

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$).

Discussion

Heat stress is one of the major constraints that confront poultry production in open house poultry farms, especially in tropical climates. Broiler chicks are homeothermic, maintain their body temperature within a slight range (thermo neutral zone) in which energy needs for thermoregulation are minimum and the net energy for production is maximum (Furlan and Macari, 2002). At high ambient temperatures, evaporative cooling through panting is important for heat dissipation. This leads to respiratory alkalosis which has been correlated with reduced feed consumption, growth rate and survival rate (Kutlu, 1996 and 2001).

Under the routine production conditions various types of stress are experienced by chicken such as heat/cold, transport, pre-slaughter holding, etc. Both high and low environmental temperatures stimulate the hypothalamo-hypophyseal adrenocortical axis which release glucocorticoids result of different feedback mechanisms which take place at various levels of the neuroendocrine system (Kristien *et al.*, 2005).

The results indicated that chickens under heat stress had a significant lower bodyweights than the control group, which clearly demonstrating the adverse effects of heat stress. Such observation was confirmed with that reported by (Balnave & Oliva,1991, Balnave & Gorman,1993 and Hayat *et al.*, 1999).

Farina *et al.* (2012) stated that high environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulse to the appetite center in the hypothalamus causing the decrease in feed consumption. In the same context birds in group (C), where $NaHCO_3$ was added at 2 g/kg gave consistently better ($P<0.05$) performance in terms of weight gains as compared to all other supplemental groups followed by Vit (C) group D. This may be due to the use of supplemental $NaHCO_3$ in poultry feed is beneficial as it is a supplemental source of Na+ ions as well as HCO_3 ions to replace CO_2 which had lost to greater extent during painting as a reflex to high temperature. This result are in agreement with Bonsembianate *et al*. (1988), (Gorman and Balnave, 1994), 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. Also Hassan *et al.* (2009) reported that heat exposure reduces plasma carbon dioxide and bicarbonate which may affect the blood pH and induce a nutritional requirement for bicarbonate. Moreover, supplementation of diet with Vit C can be beneficial due to its known metabolic functions assister ves as a classical enzyme cofactor and as a protective agent (Ombabi, 2004).

The normal blood pH should be close to physiological limits (pH, 7.35-7.45) (Carlson, 1997).This is necessary for themaintenance of protein structure and function, which isanessential condition fornormal 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).

Concerning, the results in this study indicated that the highest blood pH value was recorded in heat stressed group (B) compared to all other supplemented groups. These results are in agreement with Belay and Teeter (1996) and Farina *et al.* (2012) who showed that heat stress causes rapid and

shallow breathing inducing respiratory alkalosis thusin creasing blood pH. Also, Ahmad and Sarwar (2006) reported that the consequence of increased respiration rate (panting) is the ensuing respiratory alkalosis. Excessive loss of carbon dioxide (CO_2) during panting reduced the partial pressure of CO_2 in blood plasma. In turn, the bicarbonate buffer system lowered the concentration of hydrogen ions and caused a rise in plasma pH and plasma bicarbonate levels, which developed a condition known as respiratory alkalosis. While,among theother supplemented groupsthe lowestpH value was found in birds fed $NaHCO_3$ (group C) and highest values were observed in birds fed Vit E (group E).These results were similar to the results obtained by Keskin and Durgun (1997) and Farina *et al.* (2012). Also heat exposure reduces plasma carbon dioxide and bicarbonate,which may affect the blood pH and induce a nutritional requirement for bicarbonate (Teeter *et al*., 1985 and Balnave & Gorman, 1993).

Concerning the effect of heat stress on hematological parameters, the results showed a significant elevation of RBCs count in heat stressed group than control group. This result agreed to some extent with that reported by Narkkong *et al.* (2011).While, the administration of the antioxidant vitamin C or E resulted in the maintenance of RBC counts within the value obtained pre-heat stress occur. This observation goes in accordance with Whitehead and Keller, (2003), they suggested that the vitamins prevented the release of erythrocytes from their pool in the body into the peripheral circulation apparently due to the inhibitory role of vitamin C on circulating corticosteroids in chickens under stress, because decrease in vitamin C in the body tissues especially in renal and adrenal organs have been associated with corticosteroids release. Also the obtained result showed an increase in the values of PCV and hemoglobin of supplemented birds in group (D) than heat stressed one which could be referred to the protecting effect of vitamin C on the membrane integrity of the erythrocytesas reported (Candan *et al.*, 2002, Adenkola *et al*., 2010). While Harper *et al*. (1979) suggested that it may due to the role of vitamin C in increasing the absorption of iron from digestive system. In the same context, Borges *et al.* (2007) surmised that quantitative and morphological changes in blood cells were associated with heat stress and were translated by variations in haematocrit value, leukocyte counts, erythrocyte and haemoglobin contents. Also Vecereketal. (2002) reported decreased haemoglobin levels and increased total blood leukocytes counts in chicken due to gradually increasing temperature.

Regarding, the effect of heat stress in Leukogram. The result indicated that total leukocyte count (TLC) and eosinophils were significantly reduced. While a significant increase in basophils and monocytes counts than control group was observed. This finding is in agreement with the findings of Maxwell *et al*. (1990) and Joachim *et al*. (2010).

The heterophil to lymphocyte ratio has become widely accepted as a reliable and accurate physiological indicator of the stress response in chickens, because exposure to stressors causes it to increase progressively (Gross and Siegel, 1983).

Gross and Siegel (1993) suggested that reference values for the heterophil to lymphocyte ratio of about 0.2,0.5 and 0.8 are characteristic of low, optimal and high degrees of stress, respectively. The results showed that, a significant decrease in lymphocyte, while heterophil/lymphocyte ratio increased from 0.59 to 0.71.this result agreed with Joachim *et al.* (2010).

Heat-stressed group showed significantly higher H:L ratio at 2, 4 and 6 weeks of age compared to all supplemented groups. This suggests that feed supplements may have played a role in ameliorating the heat stress-induced changes in H:L ratio. These data suggest that heat stress had adverse effect on the level of natural agglutinin of chicks, which in general indicates impairment of their humeral immune response. Results of this study agree with that of Bartlett and Smith (2003) and Siegel (1989) who reported that heat stress adversely affects immune function and impedes with disease resistance. Nworgu *et al.* (2007) and Minka & Ayo (2008) reported stability in the values of TLC and H/L ratio after treatment with vitamin C and antioxidant was observed.

Antibodies titer against N.D and A.I were greatly affected by heat stress and the result showed the dietary supplement significantly increased antibody titer of ND and AI on day 12 post vaccination ($P<0.05$). However, dietary vitamin E had higher effect on antibodies titer compared other groups, this result agreed with Saud *et al*. (1999) who reported that, high environmental temperature increases the susceptibility of chickens to infectious diseases. Suppression of antibody synthesis occurred when the birds were stressed shortly before or after immunization, this phenomenon was called "high environmental temperature-mediated immunosuppression" (HTS). Also Swenson and Reece, (1996), Berne and Levy, (1998) reported that lymphocytes are non granulated leukocytes formed in lymphoid tissues. They play an important physiological role in immunity, particularly for the production of antibodies. One of the physiological responses of exposure to stress is the release of glucocorticoids, causing dissolution of lymphocytes in lymphoid tissues and leading to lymphopenia. However, there is an increase in heterophil release by the bone marrow, thus increasing their number in circulation, but their phagocytic and bactericidal activities are decreased. Administration of feed supplement generally increased immune response under heat stress condition, with best result obtained in Vit E supplement group. This result agreed with Hasret *et al*. (2009) and result of weight lymphoid organ index, while disagreed with Farina *et al*. (2012) who

found hemagglutination Inhibition (HI) titers were higher (P<0.05) when birds were fed on diets containing $NaHCO_3$.

A noticeable decrease (P<0.01) in serum total protein was found in heat stressed group as compared to the control. These results are in line with the findings of Wajid *et al.* (2002). In other hand the result showed that feed supplements had positive effect on total protein, albumin and globulin under heat stress condition which regained around normal parameter compared with control group.These results agreed with Hassan *et al.* (2009) and Majekodunmi *et al*. (2013).

The result showed that increase values of serum glucose observed for heat stressed birds when compared with control one. This result agreed with that reported by Borges *et al*. (2007)who found an increase in glucose concentration was one of the direct responses of birds to greater adrenaline, noradrenalin and glucocorticoid secretion in stressful conditions which was needed to prepare birds for a "fight and flight" response. Kristien *et al*. (2005) reported that the stress-induced release of glucocorticoids stimulates hepatic gluconeogenesis. Reduced insulin sensitivity of peripheral tissues by glucocorticoids may also contribute to hyperglycemia.

Birds maintained at high temperatures showed significantly (P<0.01) lowered levels of Na+ and K+ as compared to control (Group A). Similar results were found by Wajid *et al* . (2002), in contrast Cheveille (1979 stated that the blood electrolytes level of chickens is altered during heat stress. While the amounts of Na+ and K+ were unchanged in broilers within each group. On the other hand researchers have generally reported are ductionin plasma levels of $K^+$and Na^+ (Deyhim *et al.,*1990 and Borges, 1997) due to heat stress, probably as a resul to f hemodilution following increased water consumption.

Regarding, the results showed that, serum, uric acid, urea, AST and ALT increased significantly ($p\leq0.05$) and total proteins, albumin, globulin decreased significantly ($p \leq0.05$) in comparison with respective control mean values similar finding was reported by Nalini *et al.* (2008).

Bogin *et al*. (1996) demonstrated significant differences in glucose levels between heat-stressed surviving and non-surviving groups of broilers. The lower levels in non-surviving chickens can be explained by possible reduced gluconeogenic activity due to the accumulation of organic acids in response to alkalosis. The increase in uric acid, · which is the main end product of nitrogen metabolism, could indicate the renal insufficiency as the secondary result of heart function insufficiency. Also Kolb(1984) observed that blood

glucose concentration is directly responsive to an increase in glucocorticoids during stress.

It could be concluded that heat stress adversely affect birds body performance, blood indices, immunity and biochemical parameters. Significant improvement in the performance and immune response of chronically heat-stressed broiler chicks given the feed supplements, Ascorbic acid supplementation enhanced the hematological profile of birds while feed supplements with Vit E improve immunity against vaccination but generally, $NaHCO_3$ being slightly superior to Vitamin C and Vitamin E.

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مقارنة كفاءة بعض المواد المستخدمة للحد من الاجهاد الحرارى واثرها على الاداء العام والمناعة وبعض القياسات البيوكيماوية في بداري التسمين

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اجريت هذه الدراسة لمقارنة وتقييم آثار اضافات الاعلاف بيكربونات الصوديوم (NaHCO3)، وحمض الأسكوربيك (فيتامين سى) وفيتامين (هـ) على معدل الاداء والجهاز المناعي والتغيرات البيو كيميائية فى دم الطيور المعرضة للاجهاد الحرارى لفترات طويلة. اجريت الدراسة على عدد مائتي كتكوت تسمين عمر يوم قسمت الى ٥ مجموعات كل مجموعة تحتوى على ٤٠ طائر وكانت الاولى ضابطة بدون اضافات ، المجموعة الثانية معرضة لاجهاد حرارى وبدون اضافات، المجموعة الثالثة اعطيت بيكربونات الصوديوم بمعدل ٢ جم / كجم علف ، والمجموعة الرابعة اعطيت فيتامين سى ، بمعدل ٢٠٠ ملجم / كجم علف والخامسة اعطيت فيتامين هـ ، بمعدل ٣٠٠ ملجم / كجم علف. وقد تم تعريض جميع الطيور باستثناء المجموعة الاولى لدرجات حرارة تراوحت من ٣٥ الى ٤٠ درجة مئوية من عمر يوم إلى عمر ٦ أسابيع بمعدل ٦ ساعات يوميا. تم أخذ عينات في ٢،٤ و ٦ أسابيع من العمر.وقد أظهرت النتائج زيادة معنوية فى الوزن لجميع المجموعات المعالجة مقارنة بالمجموعة الضابطة مع استجابة أفضل في الطيور التي غذيت على بيكربونات الصوديوم (NaHCO3) ، وكانت هناك زيادة فى نسبة أوزان غدة فابريشى والغدة الصعترية والطحال الى وزن الجسم ، وهو مؤشر يدل على المناعة الخلطية بينما كانت نسبة الخلايا متغيرة الحبيبات / الخلايا اللمفاوية ، وهو مؤشر على يدل على الإجهاد منخفض لجميع المجموعات مقارنة بالمجموعة الضابطة . وقد وجد انخفاض الأجسام المضادة بعد التحصين لمرض النيوكاسل والانفلونزا باستخدام اختبار مانع التلازن للمجموعة المعرضة للإجهاد الحرارى عن المجموعة الضابطة بينما وجد تحسن ملحوظ فى مستوى الاجسام المناعية للمجموعات ذات الاضافات الغذائية المختلفة عن المجموعة الضابطة وكانت المجموعة التى تعاطت فيتامين هـ اعلى تأثيرا من غيرها فى مستوى الاجسام المناعية. وقد وجد زيادة معنوية فى مستوى حمض اليوريك في مصل الدم وكذلك اليوريا والجلوكوز وانزيمات الكبد في حين انخفضت معنويا مستويات الصوديوم والبوتاسيوم فى الدم وكذلك البروتينات الكلية والألبيومينو الجلوبيولين، عن المجموعة الضابطة ولم تكن هناك فروق معنوية لمجموعات التى تعاطت بيكربونات الصوديوم (NaHCO3)، وحمض الأسكوربيك فيتامين (سى) وفيتامين (هـ) على نواتج الأيض البلازما من ألانينامينو ترانسفيريزواسبارتيت امينو ترانسفيرز.

وبصفة عامة ، أشارت النتائج إلى تحسن كبير في الأداء والاستجابة المناعية للدجاج التسمين المعرض للاجهاد الحرارى المتعاطى لمكملات الأعلاف، وان تناول مكملات حمض الأسكوربيك قد اثر ايجابيا على مكونات الدم ولكن المجموعة المعالجة ببيكربونات الصوديوم اظهرت تفوقا قليلا عن فيتامينسي وفيتامين هـ في باقى المعاملات.