EFFECT OF DIETARY ASCORBIC ACID AND BETAINE ON RECOVERY FROM HEAT STRESS ADVERESE EFFECTS IN SLOW-GROWING CHICKS IN THE SUBTROPICS

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SUMMARY

This research was conducted to study the effect of dietary ascorbic acid (AA) and Betaine (Bet) to relief the adverse effects of heat stress (HS) on performance of slow-growing chicks in the subsubtropics. Three hundreds 21-d old chicks from El-Salam strain were randomly divided among 5 treatments (each of 5 replicates of 12 unsexed chicks each) and housed in floor pens. One group was kept under thermoneutral condition at 28± 4°C and 55±3% relative humidity (RH) during 21-84 d of age and fed practical corn-soybean meal basal diet (positive control). The other four groups were kept for three successive days weekly under HS at 38±1.4°C and 49±2% RH from 12.00 to 16.00 pm. Chicks in HS treatments were fed basal diet without (negative control), with 250 mg AA/kg diet or Bet at 0.5 and 1g/kg diet. HS decreased ($p<0.05$) body weight gain, feed intake, blood glucose and total protein contents, humoral immune competence to sheep red blood cell (SBRCs), dressing, liver and giblets percentages and meat water holding capacity (WHC); impaired feed conversion (FC) and economic efficiency (EE, 33%); and increased meat dry matter and blood Ca and triglycerides contents. Adding AA and Bet into basal diet alleviated ($P<0.05$) the HS adverse effects on growth, feed intake, FC, protein digestibility and dressing, liver and giblets percentages. Also, a complete recovery from the adverse effects on plasma glucose and partial recovery ($P<0.05$) was observed in total protein, triglycerides, blood pH, packed cell volume (PCV), hemoglobin (Hb), immune competence to SBRCs, rectal temperature (RT) and respiration rate (RR). Bet at 1g/kg and AA at 250 mg/kg diets was equally potent for partial relief of HS effects on the performance and EE (27%) for slow-growing chicks.

Keywords: Ascorbic acid, betaine, heat stress, chickens, performance, physiological

INTRODUCTION

In the subtropics, HS is a major problem that adversely affects chickens performance and physiological traits. The effect of HS on fast chickens was extensively investigated; but, little attention was given to the slow-growing chicks, even though they contributed to ~30% of meat and egg production in the tropical and subtropical areas. Generally, stressors induce many physiological, endocrine and productive responses (Attia *et al*., 2006; Daghir, 2008). The effect of HS on birds

involves two major factors: feed/nutrient intake and metabolic modifications (Gous and Morris, 2005; Yahav *et al*., 2005; Attia *et al*., 2006; Lin *et al*., 2006). During HS, animal cells attempt to hold water by accumulating ions such as K. The increase in K concentration and other ions generally have adverse effects on the metabolic process (Graham, 2002; Mujahid *et al*., 2005). Feeding AA to birds exposed to HS might be beneficial (Pardue *et al*., 1985; Lin *et al*., 2006), as demonstrated by the reduction in plasma corticosterone level (Mahmoud *et al*., 2004), adrenocorticotropic hormone (Sahin *et al*., 2003), increased serum insulin and T_3 and T_4 concentration (Sahin *et al*., 2002). Also, AA reduced respiratory rate (RR) in chicks exposed to HS by increasing fatty acid oxidation over the increase in protein-derived gluconeogenesis, increased feed intake, meat quality and yield while decreased carcass fat (Kutlu, 2001; Lin *et al*., 2006), and maintained the redox balance and immune response (Puthpongsiriporn *et al*., 2001; Lin *et al*., 2003; Al-Ghamdi, 2008). Betaine is a common term for trimethylglycine, substrate for Bet-homocysteine methyltransferase in the liver and kidney (Kettunen *et al*., 2001). When the three methyl groups were transferred to homocysteine to produce methionine, Bet become the amino acid glycine then it is metabolized as normal (Graham, 2002; Attia *et al*., 2005). Bet is often found in high concentrations in plants subjected to drought, and this is due to the water balance or osmoregulatory property caused by Bet (Kettunen *et al*., 2001; Graham, 2002). However, Bet is not present in large quantities in animal feedstuffs (Wang *et al*., 2004). In this regard, reducing HS adverse effects on performance of birds fed Bet may be due to improve cell osmoregulation (Graham, 2002). Similarly, Garcia *et al*. (2000), Wang *et al*. (2004) and Attia *et al*. (2005) observed that Bet may play important roles such as improving growth, FCR, fat distribution (Wang *et al*., 2004), immune response and coccidiostat enhancer (Swain and Johri, 2000; Kettunen *et al*., 2001; Remus *et al*., 2004). Thus, Bet is a multi-nutritional agent that may help chickens to resist poor management and HS. This work aimed at investigating the potential of using Bet to relief the adverse effects of HS on productive performance, meat quality, blood hematology, metabolic profile and humoral immune competence (SRBCs test) of slow-growth chicks used for meat production in the subtropics compared to AA as a well-known stresses relief.

MATERIALS AND METHODS

Experimental design, chicks and diets

This study was carried out at Sakha Animal Production Research Station during June-August. 2006 to study the effect of AA and Bet to relief the adverse effects of HS on slow-growing chicks in the subtropics. Three hundreds, 21-d old unsexed chicks of slow-growing (El-Salam strain, white feathers crossbred) *gallus gallus F. Domestica* originated from mating Nicholls×Mamorah [Alexandria {Fayoumi× Polymouth Rock×RIR×Leghorn}×Dokki$_4$ {Parred Polymouth Rock×Fayoumi]) were distributed into 5 groups of 5 replicates each (12 birds/replicate) and housed in floor pens (1×1m). One group was kept under thermoneutral condition at 28± 4°C and 55±3% relative humidity (RH) during 21-84 d of age and fed practical corn-soybean meal basal diet (positive control). The other four groups were kept for three successive days weekly under HS at 38±1.4°C and 49±2% RH from 12.00 to 16.00 pm. The heat source was provided by gas heaters supplemented with thermometer to control the temperature inside pens. The temperature was measured several times

daily during the HS period at different locations of the pens to assure normal distribution of the heat among the HS treatment pens. Chicks in HS treatments were fed basal diet without (negative control), with 250 mg AA (a heat stabilized product produced by Hoffmann-La Roche) /kg diet or Bet (natural Betafin® S6, Danisco Animal Nutrition) at 0.5 and 1g/kg diet. Diets were supplemented with anticoccidial drug (Uccma pedomix produced by Uccma Company) at 1kg/ton containing 125 g of Clopidol. This was done to avoid coccidiosis challenge and reduce the efficiency of Bet as a coccidiostat enhancer rather than its osmolytic benefits. Diets were also supplied with adequate amounts of methionine and choline to control the impact of bet as methyl donor group. Water and feed were provided *ad libitum*. Birds were individually weighed every 3 wk and simultaneous feed intake and FCR on replicate basis was also recorded. Mortality rate was daily recorded and used for correction of feed intake/b/d. At the end of the experiment, 6 birds (3 male+3 female)/ treatment were slaughtered for carcass characteristics. Chemical analyses for DM, protein, lipids, and Ca were done according to AOAC (1995) in skinless-boneless pooled samples of breast and thigh (1:1,wt:wt) muscles. Values of meat composition were expressed on DM basis. Meat characteristics as tenderness, water holding capacity (WHC), colour intensity and pH value were determined as outlined by Attia *et al.* (2003). Sheep red blood cells (SRBCs) were used as an antigen test to quantitatively analyze the humoral immune competence. Eight chicks per group were immunized i.v. via a wing vein with 1 ml SRBCs solution of 10% SRBCs suspension in sterile saline. The chicks were injected just before heat regime of the first day. At 3, 6 and 9 days post-immunization, ~2.0 ml blood samples were collected. The levels of antibody were determined using a micro hemeagglutination technique as cited by (Kai *et al*., 1988). Antibody titer values were expressed as log 2 of the highest serum dilution giving total agglutination. Biweekly rectal temperature was monitored by thermo code electric gauge with accuracy of 0.1°C. The respiration rate was measured by counting the breath/minute through observing the abdominal movement for one minute. Blood pH, hemoglobin (Hb) and PCV were determined during the 5^{th} wk old. The measurements for RR, RT, blood pH, Hb and PCV were taken on eight birds/treatment just before HS, 2 h before end of HS, and 4 h after end of HS. Heparinized blood samples was taken (~3 ml) from the brachial vein to determine pH value using digital electric by pH meter immediately after samples collection. The Hb level was estimated by cyanomethemoglobin procedure (Eilers, 1967). Heparinized blood was used for determination of PCV using Wintrobe hematocrit tubes. Blood samples were centrifuged at 4,000 rpm for 20 min then PCV values were obtained by reading the packed cell volume on the graduated hematocrit tubes. Other nine blood samples per group were also collected at 12 wk of age without or with heparin, centrifuged at 3000 rpm for 20 minutes to obtain serum and plasma, respectively, and stored at $-18C^{o}$ for further analyses. Plasma glucose (Trinder, 1969), serum total protein (Armstrong and Carr, 1964) were measured.. Plasma triglycerides were determined using Sigma Diagnostics, procedure No. 336. Serum Ca was determined by colorimetric method using available commercial kits (SCLAVO Inc., 5 Masard Count, Wayn NS 07470, USA). At the end of the experiment, 8 birds/group were housed in separate metabolic cages for 5 days. After a 3-d preliminary period, feed intake and excreta were measured and collected for 5 days. The proximate analyses of feed and dried excreta were determined according to AOAC (1995). The apparent digestibility of DM, CP, EE, CF and OM was calculated (Attia *et al*. (2006). Data

were statistically analyzed by GLM of SAS® (SAS Institute, 1990; Cary, NC, USA) using one-way ANOVA. Whereas data for blood pH, PCV, and Hb, RT and RR were analyzed using two-way factorial design, where time of sampling besides treatments were employed in the model besides HS. Before analysis, all percentages were subjected to logarithmic transformation (log10x+1) to approximate normal distribution. Mean difference ($P \leq 0.05$) was tested using Student-Newman-Keuls (SNK) Test. When a significant interaction was obtained ($P<0.05$), mean differences were compared using LSD Test.

Table 1. Composition of the experimental chicks basal diets (21-84 d old).

Ingredients [g/kg]	Growing 21-70 d of age	Finishing 71-84 d of age
Yellow corn	630.5	733
Soybean meal, 44%CP	330	230
Dicalcium phosphate	20.0	18.0
Salt	3.0	3.0
Limestone	9.0	8.0
Vit. + Min. mixture	3.0	3.0
Vegetable oil	3.0	3.5
Methionine	1.0	1.0
Lysine	0.5	0.5
Total	100	100
Calculated and analyzed values:		
Crude protein, %[b]	19.9	16.4
Crude protein, %[c]	19.5	16.1
ME, MJ /kg[b]	12.05	12.57
Calcium, %[b]	090	0.80
Available P, %[b]	0.45	0.40
Methionine, %[b]	0.42	0.37
TSAA, %[b]	0.75	0.66
Lysine, %[b]	1.10	0.85
Choline, mg/kg[b]	1310	1100
Dry matter, %[c]	89.32	90.19
Crude fiber, %[c]	3.03	2.45
Ether extract, %[c]	4.76	5.43

[a] kg of vitamin- mineral premix per ton of feed supplied each kg of diet with Vit. A 12000 IU; Vit. D3 2000 IU; vit. E. 10mg ; Vit. k_3 2mg; Vit. B_1 1mg; Vit. B_2 4mg; Vit. B_6 1.5 mg; Pantothenic acid 10mg; Vit.B_{12} 0.01mg; Folic acid 1mg; Niacin 20mg; Biotin 0.05mg; Choline chloride (50% choline) 500 mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; ethoxyqain 3000 mg.

[b] calculated values were according to NRC (1994) text book values for feedstuffs.

[c] analyzed values were according to AOAC (1995).

ME= metabolizable energy value; TSAA= total sulphur amino acids.

RESULTS

Productive performance, Digestibility coefficients

Table (2) shows the effect of different treatments on productive performance of chicks. BWG and feed intake significantly ($P<0.05$) decreased, while FCR impaired

($P<0.05$) upon exposure of chicks to HS. AA and Bet caused an increase ($P<0.05$) in BWG where 1 g of Bet was more effective ($P<0.05$) than 0.5g of Bet. AA and different levels of Bet increased ($P<0.05$) feed intake and improved FCR, without differences ($P>0.05$) from the thermoneutral group. However, the effect of treatments on FCR was not observed during 10-12 wk of age. The percentage of mortality was 0.75% of the total experimental population. The highest number was from unsupplemented HS chicks. Table (2) shows the effect of different treatments on nutrient digestibility. Only digestibility of CP increased ($P<0.05$) due to AA when compared to unsupplemented HS chicks. On the other hand, both doses of Bet had small effect ($P>0.05$) on CP digestibility. Digestibility of other nutrients was not affected by different treatments.

Table 2. Productive performance and nutrient digestibility of slow-Growing chicks as affected by heat stress and dietary AA or betaine

Wks of age	Control (+)	Heat stress treatments (-)	+AA 250 ppm	+Betaine levels 0.5 g/kg	+Betaine levels 1 g/kg	P value	SEM
Body weight gain, g/bird/period:							
3-6	369.1^{a}	309.5^{e}	344.2^{d}	330.9^{d}	355.6^{b}	0.001	2.56
7-9	373.4^{a}	328.1^{d}	375.8^{a}	350.4^{c}	367.2^{b}	0.001	2.13
10-12	380.8^{a}	358.9^{c}	374.8^{ab}	369.5^{b}	377.6^{a}	0.001	1.91
3-12	1123.3^{a}	996.6^{d}	1094.8^{b}	1050.9^{c}	1100.4^{b}	0.001	4.61
Feed intake, g/bird/period:							
3-6	1164.8	1115.4	1164.6	1139.6	1172.6	0.07	5.68
7-9	1318.1^{a}	1244.9^{b}	1333.3^{a}	1279.8^{ab}	1333.3^{a}	0.003	4.6
10-12	1549.8^{a}	1476.5^{b}	1570.0^{a}	1530.2^{a}	1560.0^{a}	0.01	5.71
3-12	4032.7^{a}	3836.8^{b}	4067.9^{a}	3949.6^{ab}	4065.9^{a}	0.008	6.49
Feed conversion ratio, g/bird/period:							
3-6	3.156^{c}	3.604^{a}	3.384^{b}	3.443^{b}	3.262^{c}	0.001	0.05
7-9	3.530^{b}	3.794^{a}	3.548^{b}	3.652^{ab}	3.611^{ab}	0.01	0.08
10-12	4.070	4.117	4.189	4.141	4.126	NS	0.04
3-12	3.590^{b}	3.851^{a}	3.716^{ab}	3.758^{ab}	3.673^{b}	0.01	0.06
Number of dead birds:							
3-12	1.0	2.0	0.0	1.0	0.0	ND	--
Apparent nutrient digestibility, %:							
DM	70.9	68.4	70.3	70.1	70.3	NS	0.75
CP	89.3^{ab}	86.4^{b}	90.0^{a}	87.9^{ab}	89.0^{ab}	0.05	0.39
EE	88.1	87.2	87.9	87.7	88.1	NS	0.27
CF	13.4	13.0	13.5	13.1	13.3	NS	0.21
OM	80.3	81.2	79.8	79.3	80.1	NS	0.34

^{abc}Means within a row not sharing a common superscript differ significantly P≤. 05; NS, not significant; ND, not done. AA= ascorbic acid

Blood pH, PCV, Hb, and metabolic profile

Table (3) shows the impact of different treatments on blood pH, PCV, Hb and metabolic profile. Only blood pH was increased ($P<0.05$) due to HS either during or

after the course of heat regimen as compared to the thermoneutral group. Whilst AA and both doses of Bet similarly led to partially recovery from HS on pH value during HS course. This effect was diminished (P>0.05) after HS time. Blood PCV and Hb of HS chicks decreased (P<0.05) during and after HS course compared to the control group. Meanwhile, AA or 1 g of Bet/kg diet improved (P<0.05) PCV and Hb compared to the negative control, and had greater effect (P<0.05) than 0.5 g Bet/kg diet during HS course. At the end of exposing time, AA had greater effect on PCV (P<0.05) than levels of Bet. Bet had a dose dependent effect (P<0.05). Blood Hb after HS was partially restored (P<0.05) due to AA and Bet. Time of sampling had a significant effect (P<0.05) on blood pH, showing higher values during HS than those before and after HS and were also higher (P<0.05) after HS than that before HS. However, the changes in blood PCV and Hb showed the opposite trend over time. There was a decrease (P<0.05) in plasma glucose and serum total protein due to HS

Table 3. Blood pH, PCV and Hb of slow-growing chicks before, during and after exposure to heat stress and supplemented with AA or betaine

Criteria	(+) Cont	Heat stress treatments				P value	SEM	Time effect
		(-)	+AA 250pp	+Betaine level 0.5g/kg	1 g/kg			
Blood pH value:								
Before exposure	7.49	7.49	7.49	7.48	7.48	0.08	0.15	7.49^{c}
During exposure	7.49^{c}	7.78^{a}	7.70^{b}	7.73^{b}	7.71^{b}	0.03	0.11	7.68^{a}
After exposure	7.49^{b}	7.59^{a}	7.57^{a}	7.58^{a}	7.56^{a}	0.04	0.17	7.56^{b}
PCV, %:								
Before exposure	33.6	33.6	33.5	33.6	33.5	0.12	0.81	33.6^{a}
During exposure	33.5^{a}	26.2^{d}	28.6^{b}	27.2^{c}	28.7^{b}	0.02	0.72	28.7^{c}
After exposure	33.6^{a}	28.3^{d}	31.0^{b}	28.7^{d}	29.9^{c}	0.01	0.64	30.3^{b}
Hb, g/100ml:								
Before exposure	8.96	8.96	8.95	8.97	8.95	0.18	0.08	8.96^{a}
During exposure	8.97^{a}	6.53^{c}	6.78^{b}	6.52^{c}	6.81^{b}	0.02	0.12	7.12^{c}
After exposure	8.97^{a}	7.51^{c}	7.78^{b}	7.71^{b}	7.72^{b}	0.03	0.09	7.94^{b}

^{abcd}Means within a row for treatments or column for time effect not sharing a common superscript differ significantly P≤ 0.05. AA= ascorbic acid

compared to the positive control whilst the contrary was shown in plasma triglyceride and serum Ca concentration. However, AA and 1 g of Bet/kg diet induced complete recovery (P<0.05) in plasma glucose and partial relief in plasma triglycerides and serum total protein. Also, 1 g Bet per kg diet completely relief the negative effect (P<0.05) of HS on serum Ca level. A dose response of Bet was observed in plasma triglyceride and serum total protein and Ca; however, both doses of Bet showed similar effect on plasma glucose.

Blood constituents, responses to SRBC's and lymphoid organs:

Table (4) shows the impact of different treatments on blood constituents, response to SRBC's and lymphoid organs There was a decrease (P<0.05) in plasma glucose and serum total protein due to HS compared to the positive control whilst the

contrary was shown in plasma triglyceride and serum Ca concentration. However, AA and 1 g of Bet/kg diet induced complete recovery ($P<0.05$) in plasma glucose and partial relief in plasma triglycerides and serum total protein. Also, 1 g Bet /kg diet completely relief the negative effect ($P<0.05$) of HS on serum Ca level. A dose response of Bet was observed in plasma triglyceride and serum total protein and Ca; however, both doses of Bet showed similar effect on plasma glucose. HS had a negative effect ($P<0.05$) on the responses to SRBCs at day 6 and 9 post-injection. Moreover, AA and 1 g of Bet /kg restored the humoral immune competence to the level of the thermoneutral group. There was a lack of effect ($p>0.05$) of HS on relative weight of bursa of fabricius, thymus, however the spleen percentage was significantly affected ($P<0.001$), with no significant differences among means based on SNK test.

Table 4. Blood constituents, response to SRBC's and lymphoid organs of slow growing chicks as affected by heat stress and dietary AA or betaine

Criteria	(+) Cont.	Heat stress treatments				SEM	P value
		(-)	+AA 250 ppm	+Betaine levels 0.5g/kg	1 g/kg		
Blood constituents:							
Plasma glucose	226.0^{a}	213.5^{c}	223.4^{a}	217.8^{bc}	222.0^{ab}	2.43	0.001
Plasma triglycer.	72.4^{d}	80.2^{a}	75.8^{c}	78.5^{b}	75.8^{c}	0.54	0.003
Serum T. protein	4.76^{a}	4.29^{d}	4.57^{b}	4.46^{c}	4.59^{b}	0.09	0.001
Serum Calcium	7.04^{d}	7.29^{a}	7.15^{bc}	7.20^{b}	7.10^{cd}	0.13	0.001
Responses to SRBCs at:							
3-days	5.00	4.50	5.13	4.80	5.25	0.07	NS
6-days	7.25^{ab}	6.25^{c}	7.25^{ab}	6.50^{bc}	7.50^{a}	0.06	0.006
9-days	4.75^{a}	3.50^{b}	4.75^{a}	3.75^{b}	4.88^{a}	0.09	0.001
Lymphoid organs,%:							
Bursa.of Fabricius	0.220	0.221	0.232	0.209	0.211	0.01	NS
Thymus	0.455	0.430	0.450	0.436	0.439	0.02	NS
Spleen	0.263	0.227	0.247	0.228	0.237	0.01	0.001

^{abcd}Means within a row not sharing a common superscript differ significantly P≤.05;
NS= not significant. AA= ascorbic acid

Rectal temperature (RT):

Table (5) shows the influence of different treatments and sampling time on RT. The RT was increased ($P<0.05$) due to HS in all groups during all times compared with the +control (natural range). However, AA led to a complete relief ($P<0.05$) of negative effect of HS only before heat exposure course. Both doses of Bet was less effective ($P<0.05$) than AA for relief of RT from HS effect before and during heat exposure course. After heat course, only 1 g of Bet was the most effective agent ($P<0.05$) for relief RT from HS effect. Also, a dose based effect of Bet was shown on RT during and after HS time. There was a time effect on RT before, during and after HS ($P<0.05$). Before HS, there were only significant differences in RT at 5 and 7 wk old and other times. During HS, the RT declined ($P<0.05$) with advanced age

of chicks, and stabilized between 7 and 9 wk then declined. The RT was decreased (P<0.05) after HS from 5 wk of age, then stabilized between 7 and 9 wk of age. The RT maximized at 5 and minimized at 12 wk of age. In general, the changes in RT overtime within treatments in responses to HS before heat exposure course was small, however, increased during and after heat course and were small in thermoneutral groups across all HS periods. The results indicate that, before exposure to HS, there were small changes in RT in groups supplemented with AA and Bet, provided that AA was the most effective. Moreover, a dose response effect was observed to Bet at 9 and 12 wk of age. However, the unsupplemented HS group exhibited the highest increase in RT. During HS time, AA and 1 g of Bet led to partial recovery from adverse effect of HS on RT and this was obvious in AA group. Moreover, 1.0 g of Bet was more potent than the low dose of it.

Table 5. Rectal temperature of slow-growing chicks before, during and After exposure to heat stress and supplemented with AA or betaine

Wks of age	(+) Cont.	Heat stress treatments				SEM	P value	Age effect
		(-)	+AA 250 ppm	+Betaine levels 0.5g/kg	+Betaine levels 1 g/kg			
Before heat exposure								
3	40.3 ^{b}	40.4^{a}	40.2^{c}	40.4^{b}	40.4^{b}	0.07	0.04	40.4^{X}
5	40.3^{c}	40.5^{a}	40.3^{c}	40.4 ^{b}	40.4 ^{b}	0.04	0.03	40.4^{X}
7	40.3^{c}	40.5^{a}	40.3^{c}	40.4^{b}	40.4 ^{b}	0.03	0.02	40.5^{W}
9	40.4^{b}	40.6^{a}	40.4^{b}	40.5^{a}	40.4 ^{b}	0.06	0.04	40.4^{X}
12	40.3^{c}	40.5^{a}	40.4	40.5^{a}	40.4 ^{b}	0.02	0.04	40.4^{X}
Mean	40.3^{C}	40.5^{A}	40.3^{C}	40.4^{B}	40.4^{B}	0.04	0.03	---
During heat exposure								
3	40.3^{c}	43.6^{a}	42.5^{b}	42.5^{b}	42.6^{b}	0.04	0.01	42.3^{W}
5	40.4^{e}	43.6^{a}	41.3^{d}	42.6^{b}	41.9^{c}	0.05	0.04	42.0^{X}
7	40.4^{e}	43.2^{a}	41.6^{de}	42.3^{b}	41.8^{d}	0.06	0.03	41.9^{Y}
9	40.5^{d}	43.0^{a}	41.6^{c}	42.4^{b}	41.7^{c}	0.09	0.04	41.8^{Y}
12	40.4^{d}	42.8^{a}	41.7^{c}	42.3^{b}	41.7^{c}	0.03	0.01	41.8^{Y}
Mean	40.4^{D}	43.2^{A}	41.7^{C}	42.4^{B}	41.9^{C}	0.07	0.02	-----
After heat exposure								
3	40.4^{c}	41.7^{a}	40.7^{b}	40.6^{bc}	40.5^{c}	0.09	0.02	40.7^{X}
5	40.5^{d}	41.6^{a}	40.9^{b}	40.6^{c}	40.6^{cd}	0.04	0.03	40.8^{W}
7	40.5^{b}	41.5	40.5^{b}	40.5^{b}	40.5^{b}	0.06	0.01	40.7^{X}
9	40.5^{c}	41.2^{a}	40.5^{c}	40.6^{bc}	40.5^{c}	0.03	0.01	40.7 ^{X}
12	40.4^{b}	40.6^{a}	40.4^{b}	40.5^{ab}	40.4^{b}	0.07	0.02	40.5^{Y}
Mean	40.4^{D}	41.3^{A}	40.6^{B}	40.6^{B}	40.5^{C}	0.02	0.01	-----

^{ABC}Means within a row (mean of treatment) not sharing a common superscript differ significantly P≤.05, based on SNK test.

^{WXY}Means within a column (age effect) not sharing a common superscript differ significantly P≤.05, based on SNK test.

^{abcd}Means within treatments within weeks (interaction effect) within each time of exposure not sharing a common superscript differ significantly P≤.05, based on LSD test. AA= ascorbic acid

In general, after termination of HS by ~4 h, there was a decline (P<0.05) in RT in the unsupplemented HS group compared to that observed during HS, however, complete relief to pre exposure period was not achieved before 12 wk of age. Obviously, there was a decline (*P*>0.05) in RT over time in the unsupplemented HS group, thereby, differences from groups supplemented with 0.5 g Bet was diminished at 12 wk of age. However, changes in RT over time were less obvious in HS groups supplemented with AA and Bet.

Respiration rate

Table (6) shows the influence of different treatments and sampling time on RR. The RR increased (*P*<0.05) due to HS in the unsupplemented group compared to the other treatment groups during all times, and maximized during heat course while minimized before heat course. However, AA induced complete relief (*P*<0.05) of RR from HS only before HS. Only 1 g of Bet was the most effective agent (*P*<0.05) for relief of RR from HS during and after HS. In general, AA and 1 g of Bet was more effective than 0.5g of Bet on relief of RR from HS especially before and during heat course. Sampling time had an effect (*P<0.*05) on RR during all exposure times. Prior to HS, there were (*P*<0.05) decline in RR with advanced age of chicks. However, differences between 5 and 7 wk of age or 9 and 12 wk of age were not significant. During HS, the RR linearly declined (*P*<0.05) as the age of birds increased then stabilized between 7 and 9 wk old. After HS, the RR decreased (*P*<0.05) after 3 wk old then stabilized between 5 and 9 wk old and declined thereafter. In general, the control groups showed small changes in RR overtime within period and in relation to HS time. There were small changes in RR in groups supplemented with AA and Bet before exposure to HS, with AA and 1 g of Bet had similar potentiality. However, HS increased RR by 42.1% compared to that of the control group. Obviously, during HS period, RR declined in the unsupplemented HS group with advanced age of chicks. The higher dose of Bet (1 g) was more effective for relief the effect of HS on RR than 0.5 g of Bet only during and after HS period. Also, after HS, both doses of Bet was more effective than AA at only 3 wk of age. After HS, RR declined (*P*<0.05) compared to that observed during HS, yet pre-exposure values were not achieved before wk 5 of age (Table 6). However, RR decreased in all HS groups and was dependent upon age of chicks, type and dose of agent. Thus, at the end of the trial, AA and thermoneutral groups were similar.

Carcass characteristics and meat quality

Table (7) shows the effect of different treatments on carcass characteristics and meat quality. Obviously, HS (*P*<0.05) reduced the percentage dressed carcasses, liver and giblets compared to the control group. AA and 1 g of Bet resulted in full recovery of the negative effects. While, 0.5 g Bet/kg diet resulted in partial recovery in liver and complete recovery in giblets. The effects of treatments on the other criteria were not significant. There was no (*P*>0.05) effect of HS and AA or Bet on CP and EE of meat, however, the effect on percentage ash tended to be significant (*P*=0.08), without differences among treatment means. However, percentage meat DM increased (*P*<0.05) of chicks exposed to HS, whilst AA and Bet resorted it (*P*<0.05) to the control level. There was no (*P*>0.05) effect of HS and AA or Bet on pH and color of meat. Nonetheless, the effect of treatments on meat tenderness approached significant (*P*=0.06) but without differences among treatment means. HS

decreased ($P<0.05$) meat WHC, whilst AA or Bet resorted ($P<0.05$) the WHC to that of the control group, and 1 g of Bet was more efficient.

Economic efficiency (EE):

Data for EE as affected by different treatments are shown in Table (8). Results indicated that HS decreased EE by about 33% compared with the control. On the other hand, addition of AA or Bet into basal diet were effective method to alleviate the adverse effect of HS on slow-growing chicks. Bet at 1ppb and AA at 250 ppm were similarly and the best additives for alleviating HS effects for EE by 27%.

Table 6. Respiration rate of slow-growing chicks before during and after exposure to heat stress and supplemented with AA or betaine

Wk of age	Contr (+)	Heat stress treatments				SEM	Age effect*
		(-)	+AA 250ppm	+Betaine levels 0.5 g/kg	+Betaine levels 1 g/kg		
Before heat exposure**							
3	55.9	56.5	56.5	56.2	56.0	0.91	56.2^{W}
5	54.4^{c}	56.0^{a}	54.6^{c}	55.3^{bc}	54.9^{b}	0.84	55.1^{X}
7	54.4^{c}	56.2^{a}	54.3^{c}	55.5^{b}	54.8^{bc}	0.71	55.0^{X}
9	54.0^{b}	56.0^{a}	54.3^{b}	55.0^{ab}	54.1^{b}	0.80	54.7^{Y}
12	53.8^{c}	56.0^{a}	53.8^{c}	55.5^{ab}	54.2^{bc}	0.68	54.6^{Y}
Mean	54.5^{C}	56.1^{A}	54.7^{C}	55.5^{B}	54.8^{C}	0.44	----
During heat exposure**							
3	55.9^{d}	82.4^{a}	63.5^{c}	71.2^{b}	61.9^{c}	0.67	71.1^{W}
5	55.2^{d}	80.2^{a}	65.4^{c}	69.8^{b}	65.1^{c}	0.69	67.1^{X}
7	54.7^{d}	77.9^{a}	64.3^{c}	69.9^{b}	64.5^{c}	0.70	66.2^{Y}
9	54.9^{d}	75.2^{a}	64.1^{c}	67.2^{b}	63.7^{c}	0.80	65.9^{Y}
12	55.1^{d}	76.0^{a}	64.1^{c}	71.6^{b}	62.9^{c}	0.71	65.0^{Z}
Mean	55.1^{E}	78.3^{A}	68.0^{C}	69.9^{B}	63.9^{D}	0.46	------
After heat exposure**							
3	55.7^{d}	60.9^{a}	60.9^{a}	57.7^{b}	56.3^{c}	0.69	58.3^{W}
5	55.2^{d}	56.9^{a}	56.1^{bc}	56.8^{ab}	55.9^{cd}	0.71	56.2^{X}
7	54.9^{d}	56.7^{a}	54.9^{d}	55.8^{bc}	55.5^{cd}	0.80	56.2^{X}
9	54.8^{d}	57.2^{a}	55.3^{c}	56.4^{b}	55.3^{c}	0.57	55.8^{XY}
12	55.1^{d}	57.5^{a}	55.6^{cd}	56.9^{b}	56.0^{c}	0.66	55.6^{Y}
Mean	55.2^{D}	57.8^{A}	56.6^{B}	56.8^{B}	55.8^{C}	0.39	------

^{ABCD}Means within a row (treatment mean) not sharing a common superscript differ significantly P≤.05, based on SNK Test.

^{WXYZ}Means within a column (age effect) not sharing a common superscript differ significantly P≤.05, based on SNK Test.

^{abcd}Means within treatments within weeks (interaction effect) within each time of exposure not sharing a common superscript differ significantly P≤.05, based on LSD Test. AA= ascorbic acid

Table 7. Carcass and meat quality traits of slow-growing chicks exposed to heat stress and supplemented with AA or betaine

Criteria	Cont. (+)	Heat stress treatments				SEM	P value
		(-)	+AA	+Betaine levels			
				0.5 g/kg	1 g/kg		
Carcass criteria, %							
Dressing	70.8[a]	68.0[b]	70.0[ab]	68.2[b]	70.8[a]	0.15	0.001
Abdom. fat	0.864	0.781	0.815	0.885	0.822	0.02	NS
Liver	2.45[a]	2.10[c]	2.36[ab]	2.26[b]	2.35[ab]	0.13	0.001
Gizzard	2.42	2.42	2.39	2.43	2.44	0.12	0.001
Heart	0.48	0.41	0.49	0.45	0.46	0.01	0.001
Giblets	5.35[a]	4.93[b]	5.24[a]	5.15[a]	5.24[a]	0.11	0.001
Pancreas	0.190	0.167	0.177	0.167	0.187	0.01	0.09
Meat chemical composition on dry matter basis, %							
DM	24.1[b]	25.8[a]	24.2[b]	24.5[b]	24.2[b]	0.31	0.01
CP	75.8	75.0	75.4	75.1	75.6	0.73	NS
EE	16.4	15.3	16.2	15.5	15.5	0.16	NS
Ash	5.00	5.10	5.00	5.00	5.10	0.19	0.08
Meat physical parameters							
pH	6.63	6.59	6.71	6.54	6.62	0.31	NS
Color	0.248	0.238	0.234	0.248	0.243	0.01	NS
Tend.,cm^2	2.36	2.32	2.38	2.31	2.42	0.11	0.06
WHC,cm^2	5.66[a]	5.43[b]	5.61[ab]	5.54[ab]	5.75[a]	0.43	0.007

[a,b,c]Means within a row not sharing a common superscript differ significantly (P≤. 0.05), NS= not significant AA= ascorbic acid

Table 8. Economic efficiency (EE) of slow-growing chicks exposed to heat stress and supplemented with AA or betaine during 21-84 d of age

Criteria	(+) Cont	Heat stress treatments				P value
		(-)	+AA	+Betaine level		
			250ppm	0.5 g/kg	1 g/kg	
Feed cost, LE	7.66[b]	7.29[c]	7.80[a]	7.55[b]	7.83[a]	0.003
Total cost, LE	10.66[b]	10.29[c]	10.80[a]	10.55[b]	10.83[a]	0.007
Total revenue, LE	13.86[a]	12.35[c]	13.52[ab]	13.00[b]	13.58[ab]	0.004
Net revenue, LE	3.20[a]	2.06[d]	2.72[b]	2.45[c]	2.75[b]	0.012
EE	0.30[a]	0.20[d]	0.25[b]	0.23[c]	0.254[b]	0.004
Relative EE, %	100	66.73	83.3	76.7	87.75	----

[Abcd] Means within a row for treatments not sharing a common superscript differ significantly P ≤0.05.
Fixed cost per chick = 3 LE, Price of kg live body weight =12 LE

DISCUSSION

The Bet contents of corn and soybean meal were reported to be below the detectable level (Chendrimada *et al.,* 2002), thus Waldroup and Fritts (2005) observed that Bet could not be detect in corn-soybean meal diet determined in

specialized Lab. On the other hand, data related to AA in animal feedstuffs are rare. Although poultry can synthesis AA from glucose, furthermore this process is not adequate under HS condition and supplementation seems necessary (Lin *et al*., 2006; Daghir, 2008; Al-Ghamdi, 2008). The negative impact of HS on broilers and layers is well known, and the response to HS was affected by exposure period, temperature, genotype and age of birds (Yahav *et al*., 2005; Attia *et al*., 2006; Lin *et al*., 2006; Daghir, 2008; Al-Ghamdi, 2008). In the present work, HS decreased growth (-11.3%), feed intake (-4.9%); digestibility of CP (-3.2%), dressing (-4%) and impaired FCR (+7.3%). Negative effects were also shown in liver weight, moisture, tenderness and WHC of meat. Furthermore, physiological traits were correlated with increasing RT and RR e.g. Correlation between RT during HS vs. pH during HS was r=0.77 (P=0.0001) and after HS r=0.67 (P=0.0003), Hb during HS was r=-0.70 (P=0.0001) and after HS r=-0.71 (P=0.0001) and PCV during HS was r=-0.75 (P=0.0001) and after HS r=-0.73 (P=0.0001). Meanwhile the negative effect of HS on serum glucose, serum total protein, plasma triglycerides and serum Ca was -5.5, -9.7, +10.8 and +3.6%, respectively. Furthermore, correlation between RT (during HS and after HS, respectively) and the aforementioned parameters was (r=-0.51, P=0.009; r=-0.45, P=0.02), (r=-0.67, P=0.0003; r=-0.64, P=0.0006), (r=0.58, P=0.002; r=0.50, P=0.01) and (r=0.59, P=0.002; r=0.75, P=0.0001), respectively. Similar values of correlation were noticed between the aforementioned criteria and RR, too. It is worth noticing that RT and RR were highly correlated during and after HS e.g. (r=0.74, P=0.0001; r=0.63, P=0.0008, respectively) and (r=0.57, P =0.003; r=0.41, P=0.04, respectively).

Obviously, the increase in RT and RR was more drastic during HS rather than before and after HS (Tables 5 and 6). The increase in RT and RR are in line with those reported by Lin *et al.* (2003; 2006) and Al-Ghamdi (2008). Additionally, there was a reduction of 13.8% and 26.3% in humoral immune competence (SRBCs response) due to HS at 6 and 9 d respectively. Whilst, mortality was very low and was not relevant to dietary treatments. Nonetheless, physiological and behavior effects of HS on chickens such as increasing RR, refusal of feed and laying down in the floor was shown during HS regime. Scant literature is available about the influence of Bet (non-protein amino acid) as an osmoregulatory agent for relief the adverse effect of HS (Wang *et al*., 2004), irrespective of its role as a methyl group donor (Türker *et al*., 2004; Wang *et al*., 2004; Attia *et al*., 2005; Hassan et al., 2005), coccidiostat and immune enhancer (Keuttunen *et al*., 2001b; Remus *et al*., 2004) compared to the well-known positive effect of AA (Gous and Morris, 2005; Lin *et al*., 2006). Results indicate that 1 g Bet/ kg diet was as effective as AA for relief the adverse effects of HS on growth (10.4 vs. 9.9%), feed intake (5.97 vs. 6.02%) and FCR (4.6 vs. 3.51%); however, it was less effective than AA for improving CP digestibility (3.0 vs. 4.2%). These reveal that 1g Bet/kg diet could alleviate the negative effect of HS on performance of slow-growing chick. The mechanism involved was suggested to be e.g. antioxidative properties that decrease tissue damage arising from lipid peroxidation resulting from cell membrane damage at HS (Whitehead and Keller, 2003; Gous and Morris, 2005; Mujahid *et al*., 2005). Meanwhile, the effect of Bet may be due to its role as osmoregulatory agent that increases water retention, methyl group donor and immune enhancer (Graham, 2002; Attia *et al*., 2005; Hassan *et al*., 2005). A dose dependent effect of Bet was observed on productive performance as 0.5 g Bet was less potent than 1 g Bet/kg diet, and AA, too. In this regard, Wang *et al*. (2004), Attia *et al*. (2005) and Hassan *et al*. (2005)

showed that Bet significantly improved performance of chicks fed methionine or choline-adequate diets.

The improved growth was concurred with decreasing RT before (r=-0.39; P=0.06), during (r=-0.73; P=0.0001) and after HS (r=-0.87; P=0.0001), and RR only during (r=-0.67; P=0.0002) and after HS (r=-0.46; P=0.02). Thus, the improved productive performance related to AA and Bet could be attributed to the reduction in RR and RT. Obviously, these agents showed much greater influence during HS time and with AA and 1 g of Bet. It was also concluded that the effect of AA and 1 g of Bet during the HS time had different influences on RT (34.7 vs. 30.1%) and RR (13.2 vs. 18.4 %). The reduction in RT and RR of AA and Bet supplemented-chicks was associated, as mentioned above, with a decrease in the blood pH and an increase in the PCV and Hb (Table 4). It is evident that the increase in blood pH value due to HS led to a further respiratory alkalosis and a reduction in the performance of chickens (Lin *et al*., 2006; Daghir, 2008). The impact of AA on performance and physiological traits was frequently reported (Gous and Morris, 2005; Lin *et al*., 2006), and was ascribed to increase bird's appetite resulting in increased feed intake (Table 2) which was in line with the review by Lin *et al.* (2006). Moreover, AA reduces the RT and RR in HS broilers (Lin *et al*., 2006), which confirmed by the present findings (Table 6). The positive influence of 1 g Bet /kg diet observed herein could be due to increasing feed intake (Table 2), improving physiological criteria (Table 4), reducing RT (Table 5) and RR (Table 6) whilst increasing water retention, as evident by increasing tenderness, WHC and moisture of meat (Table 7).

In general, the present findings indicate that, HS slow-growing chicks exhibited a decrease in humoral immune competence using SRBCs test. This is in line with results reported by (Lin et al., 2000; Mashaly *et al*., 2004; Al-Ghamdi, 2008). Meanwhile, this negative effect was alleviated by AA and 1 g of Bet/kg diet from 6 d post- injection (Table 5). There was also an increase in serum total protein and Hb (Table 4), suggesting an improvement in humoral immunity. The improvements in humoral immunity due to AA and Bet are in line with those of Swain and Johri (2000), Remus *et al.* (2004) and Attia *et al*. (2005). AA is an important antioxidants in biological system and immune response and could control the adverse effect of HS on immunological competence (Puthpongsiriporn *et al*., 2001; Lin *et al*., 2003; Gous and Morris, 2005; Lin *et al*., 2006). Relative weight of dressed carcass, liver and giblets was restored due to AA and 1 g Bet. The increase in dressing percent parallels the increase in protein digestibility. Also, Virtanen and Rosi (1995), Wang *et al*. (2004) and Attia *et al*. (2005) indicated that Bet addition to poultry diets improved breast meat yield. The increase ($P<0.05$) in carcass yield due to Bet could be attributed to its osmoregulatory effect, which increased water retention (Esteve-Garcia and Mack, 2000; Garcia *et al.,* 2000). This agree with the present findings that Bet decreased ($p<0.05$) DM of muscle and increased WHC (Table 7). Also, Kutlu (2001) found that AA improved carcass quality and yield of broilers. Obviously, HS had undesirable effects on pH, PCV and Hgb before, during and after of HS. However, the effect was greater during HS, nonetheless 4 h post HS was not sufficient to restore the values to the pre exposure values (Table 4). The decrease in growth was 16.1, 12.1 and 5.8 % at 6, 9 and 12 wk of age, showing better physiological acclimatization overtime to HS. These indicate that the negative impact of HS was substantially decreased ($P<0.05$) with progressing age of chicks, suggesting a change in metabolic profile (Lin *et al*., 2006). In this regard, Yahav *et*

al. (2005) and Lin *et al*. (2006) concluded that HS at 36° for 24 h at 3 and 5 d of age seemed to be effective method of enhancing HS resistance. In conclusion, addition of 250 mg AA or 1 Bet/kg diet for slow-growing chicks gained (14.6/g/d) during 21-84 d of age could partially alleviate the adverse effects of HS on physiological traits and productive performance.

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تأثير حامض الاسكوربيك والبيتايين في العلف على الاستشفاء من الآثار السيئه للإجهاد الحراري في الكتاكيت بطيئة النمو في المناطق شبه الاستوائيه

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أجرى هذا البحث لدراسة تأثير حامض الاسكوربيك والبيتايين في العلف لتخفيف الآثار السيئه للإجهاد الحراري على أداء الكتاكيت بطيئة النمو في المناطق شبه الاستوائيه، واستخدم في الدراسه عدد ٣٠٠ كتكوت غير مجنس عمر ٢١ يوم من سلاله السلام قسمت عشوائيا إلى ٥ مجموعات كل منها في ٥ مكررات بكل مكرره عدد ١٢ كتكوت ربيت في حجرات أرضيه، وعرضت المجموعه الأولى (كنترول موجب) إلى الظروف الطبيعيه من درجه حراره (٢٨ ±٤ ْم) و رطوبه نسبيه (٥٥ ±٣%) وغذيت على علف الأساس الذي تم تكوينه على أساس الاذره الصفراء وكسب فول الصويا خلال فتره التجربه (٢١-٨٤ يوم من العمر)، أما الأربع مجاميع الأخرى فقد عرضت لظروف الإجهاد الحراري عند درجه حراره ٣٨ ±١.٤ ْم و رطوبه نسبيه قدرها ٤٩ ±٢% لمده ٣ أيام متتاليه أسبوعيا من الساعه الثانية عشر ظهرا حتى الرابعة عصرا، وغذيت على علف الأساس بدون (كنترول سالب) أو مع اضافه حامض الاسكوربيك بمعدل ٢٥٠ملجم/كجم أو البيتايين بمعدل ٥. و١جم/كجم علف خلال فتره التجربه، وأشارت النتائج إلى أن الإجهاد الحراري أدى جوهريا إلى انخفاض معدل النمو والعلف المأكول ومحتوى الدم من الجلوكوز والاستجابه المناعيه ضد كرات الدم الحمراء للغنم ونسب كل من التصافي والكبد والحلويات وقدره العضلات على الاحتفاظ بالماء كما سجلت تدهورا في الكفاءه ألاقتصاديه بمقدار ٣٣% وارتفاعا في نسبه الماده الجافه بالعضلات ومحتوى الدم من الكالسيوم والجلسريدات الثلاثيه، وكانت اضافه حامض الاسكوربيك و البيتافين بمستوييه إلى العلف فاعله جوهريا في تخفيف الآثار السيئه للإجهاد الحراري على كل من النمو والعلف المأكول والكفاءه الغذائيه والاقتصاديه ومعاملات هضم البروتين ومكونات الدم واللحم والمناعه ضد كرات الدم الحمراء للغنم ومعدل التنفس ودرجه حراره الجسم. وتخلص هذه الدراسه إلى أن اضافه البيتافين بمعدل ١ جم/كجم أو حامض الاسكوربيك بمعدل ٢٥٠ ملجم /كجم إلى العلف سجلت أفضل الإضافات فقد كان لهما تأثيرا متقاربا وجوهريا في تخفيف الآثار السيئه للإجهاد الحراري على الأداء والكفاءه ألاقتصاديه (٢٧%) في الكتاكيت بطيئة النمو.