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ORIGINAL PAPER

Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: Effect of ascorbic acid and different levels of betaine

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Abstract Three hundreds, 21 d-old slow-growing chicks were randomly divided among 5 treatments, of 5 replicates each. Each replicate contained 12 unsexed chicks housed in (1×1) a floor pen. A group was kept under thermoneutral condition at 28±4°C and RH was 55±3% during 21–84 d of age (positive control) and fed corn-soybean meal diet. The other four groups were kept for three successive days per week under heat stress (HS) at 38±1.4°C and 49±2% RH from 12.00 to 16.00 pm. Chicks in HS treatments were fed corn-soybean meal diet without (negative control) or with 250 mg AA/kg diet and Bet at 0.5 and 1 g/kg diet. HS decreased productive performance, increased ($P<0.05$) meat dry matter, plasma triglyceride and serum calcium whereas decreased ($P>0.05$) plasma glucose, serum total protein and water holding capacity (WHC) of meat. AA and 1 g of Bet/kg diet was equally potent for partial relief ($P<0.05$) of the negative effect of HS on growth, increased ($P<0.05$) feed intake, protein digestibility ($P<0.05$), dressing out percentage, liver and giblets, whilst improved ($P<0.05$) feed conversion ratio (FCR). Also, a complete recovery from the negative effect ($P<0.05$) of HS shown on plasma glucose and partial recovery ($P<0.05$) observed in total protein, triglyceride, blood pH, packed cell volume (PCV), hemoglobin (Hgb), rectal temperature (RT) and respiration rate (RR) and improved humoral immune competence to sheep red blood cell (SBRCs) test.

Keywords Ascorbic acid · Betaine · Heat stress · Productive and physiological traits

Abbreviations

AA	ascorbic acid
BWG	body weight gain
Bet	Betaine
Ca	calcium
CA	crude ash
CP	crude protein
CF	crude fibre
D	day
DM	dry matter
EE	ether extract
FCR	feed conversion ratio
Hgb	hemoglobin
hr	hour
HS	heat stress
LSD	least significant difference
NRC	National Research Council
OM	organic matter
pH	hydrogen power

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P	probability level
RT	rectal temperature
RR	respiration rate
SAA	sulphur amino acid
SD	standard deviation
SRBCs	sheep red blood cells
wk	week
WHC	water holding capacity

Introduction

In the tropics, heat stress (HS) is a major problem that adversely affects performance and physiological traits of chickens. The effect of HS on growth of chicks was extensively investigated; however, 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 area.

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 Morriss 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 a negative effect on the metabolic process (Graham 2002; Mujahid et al. 2005).

AA addition to chicks 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), and increased serum insulin, T_3 and T_4 concentration (Sahin et al. 2002). AA reduced the 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 (Bet) 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 and 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, Bet was found to reduce the negative impact of heat stress on growth performance and survival of birds which might be attributed to improved 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 tropics compared to the ascorbic acid as a well-known stresses relief.

Materials and methods

Experimental design, chicks and diets

Three hundreds, 21-day old unsexed chicks of slow growing (El-Salam strain, a white feathers crossbred) *gallus gallus F. Domestica* originated from mating Nchols × Mamorah [Alexandria {Fayoumi × Polymouth Rock × RIR× Leghorn}× $Dokki_4$ {Parred Polymouth Rock× Fayoumi]). Chicks were weighed and equally distributed among five groups of five replicates each; each replicate (1×1 m) consisted of 12 birds. During 21–84 d of age, the positive control group was kept under thermoneutral condition in semi-opened house, the temperature during the experimental period ranged from 25 to 11.6, 41.3 to 22.2 and 29.5 to 18.8°C during 1–28, 29– 56 and 57–84 d of age respectively. The corresponding relative humidity ranged from 95.5 to 33.1, 40.2 to 11.9 and 95.9 to 38.3%, respectively. Thus the average temperature during the experimental period was 28±4°C and RH was 55±3%. During the same period (21–84 days), the other four groups were kept for three

successive days per week under 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. The temperature was monitored several times daily during the HS period at different locations of the pens to homogenous normal distribution of the heat among the HS treatment pens. These chicks were fed diet without (negative control) or with 1 g Vitamin C (containing 250 mg AA; a heat stabilized product produced by Hoffmann-La Roche) or 0.5 and 1 g/kg diet of Bet (natural Betafin® S6, Danisco Animal Nutrition). Corn-soybean meal diets Table (1) were fed in the growing and finishing periods from 21 to 70 and 71 to 84 d of age, respectively. The experimental diets were supplemented with anticoccidial drug (Uccma pedomix produced by Uccma Company) at 1 kg/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. Chicks were kept in floor pens furnished with rice hulls. Water and feed were provided *ad libitum*.

Table 1 Composition and calculated analyses of the diets fed during 21–84 d of age

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 (premix)[a]	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 value		
CP,%[b]	19.9	16.4
CP,%[c]	19.5	16.1
ME MJ /kg[b]	12.05	12.57
Ca,%[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
DM,% [c]	89.32	90.19
CF,[c]	3.03	2.45
EE,[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. 10 mg; Vit. k3 2 mg; Vit. B1 1 mg; Vit. B2 4 mg; Vit. B6 1.5 mg; Pantothenic acid 10 mg; Vit.B12 0.01 mg; Folic acid 1 mg; Niacin 20 mg; Biotin 0.05 mg; Choline chloride (50% choline) 500 mg; Zn 55 mg; Fe 30 mg; I 1 mg; Se 0.1 mg; Mn 55 mg; ethoxyqain 3000 mg.

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

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

CP, crude protein; ME, metabolizable energy value; TSAA,total sulphur amino acids;

DM, dry matter; CF, crude fibre; EE, ether extract

Data collection

The 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/chick/day. At the end of the experiment (12 wk of age), 6 birds of each treatment, as three of each sex, were slaughtered for carcass characteristics. Chemical analyses for DM, protein, lipids, and CA were done according to AOAC (1995) in skinless-boneless pooled samples (50:50; weight/weight) of breast plus thigh muscle. Values of meat composition were expressed on dry matter basis. Meat quality measurements such as meat tenderness and water holding capacity (WHC), meat colour intensity and pH value were determined as outlined by Attia (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

(Hgb) and PCV were determined during the 5th wk of age. The measurements for RR, RT, blood pH, hemoglobin (Hgb) 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 collection of samples. Hemoglobin concentration (Hgb) was detected as g/dL by the cyanomethemoglobin procedure (Eilers 1967). Heparinized blood was used for determination of PCV using Wintrobe hematocrit tubes. Blood sample were centrifuged for 20 min at 4,000 rpm then PCV values were obtained by reading the packed cell volume on the graduated hematocrit tubes.

Nine blood samples per treatment were collected at 12 wk of age without or with heparin to obtain blood serum and plasma respectively. Plasma and serum were obtained by centrifugation of blood at 3000 rpm for 20 minutes, and stored at − 18°C for further analyses. Plasma glucose concentration (mg/100 ml) was determined by the method of Trinder (1969). Serum total protein (g/100 ml) was measured by the Biuret method as described by Armstrong and Carr (1964). Plasma triglycerides were determined using Sigma Diagnostics, procedure No. 336. Serum Ca was determined colorimetric method using available commercial kits (SCLAVO Inc., 5 Masard Count, Wayn NS 07470, USA).

At the end of the experiment (12 wk of age), eight birds from each group were housed in separate metabolic cages for 5 days. After a 3 days preliminary period, feed intake and excreta were measured and collected during 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 according to Attia et al. (2006).

Statistical evaluation

Data were analyzed by GLM of SAS® (SAS Institute 1990; Cary, NC, USA) using one-way model. Whereas, data for blood pH, PCV, and Hbg, rectal temperature and respiration rate were analyzed using two-way design: Before analysis, all percentages were subjected to logarithmic transformation ($\log10\ x + 1$) to approximate normal distribution. Mean difference at $p \leq 0.05$ was tested using Student-Newman-Keuls-Test. When a significant interaction p value was obtained (<0.05), mean differences were compared using LSD.

Results

Productive performance

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 0.5 g 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.

Digestibility coefficients

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.

Blood pH, PCV, Hgb, and metabolic profile

Table (3) shows the impact of different treatments on blood pH, PCV, Hgb and metabolic profile. Only blood pH was increased ($P<0.05$) due to HS either during or after the course of heat regime as compared to the thermoneutral group. Whist AA and both doses of Bet similarly led to partially recovery from HS on pH value during only HS course. This effect was diminished ($P>0.05$) after HS time. Blood PCV and Hgb 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 Hgb compared to the negative control, and had greater effect ($P<0.05$) than 0.5 g Bet/kg diet during HS course.

Table 2 Productive performance, number of dead birds and apparent nutrient digestibility of slow- growing chicks exposed to heat stress and supplemented with ascorbic acid and different levels of betaine (Mean± SD)

Wk of age	Control	Heat stress treatment			P value
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g /kg	
Body weight gain, g/bird/period					
3–6	369.1^{a}±14.5	309.5^{e}±10.3	344.2^{d}±11.7	330.9^{d}±9.5	355.6^{b}±10.4	0.001
7–9	373.4^{a}±20.7	328.1^{d}±14.1	375.8^{a}±15.0	350.4^{c}±16.1	367.2^{b}±16.2	0.001
10–12	380.8^{a}±19.1	358.9^{c}±12.5	374.8^{ab}±22.1	369.5^{b}±15.4	377.6^{a}±19.8	0.001
3–12	1123.3^{a}±20.4	996.6^{d}±8.6	1094.8^{b}±19.6	1050.9^{c}±12.5	1100.4^{b}±15.6	0.001
Feed intake, g/bird/period						
3–6	1164.8±48.2	1115.4±36.2	1164.6±5.6	1139.6±11.4	1172.6±5.7	0.07
7–9	1318.1^{a}±34.2	1244.9^{b}±41.3	1333.3^{a}±6.3	1279.8^{ab}±35.2	1333.3^{a}±10.3	0.003
10–12	1549.8^{a}±39.3	1476.5^{b}±45.7	1570.0^{a}±9.1	1530.2^{a}±40.0	1560.0^{a}±7.7	0.01
3–12	4032.7^{a}±119.4	3836.8^{b}±113.5	4067.9^{a}±9.1	3949.6^{ab}±83.6	4065.9^{a}±5.7	0.008
Feed conversion ratio, g/bird/period						
3–6	3.156^{c}±0.101	3.604^{a}±0.117	3.384^{b}±0.008	3.443^{b}±0.013	3.262^{c}±0.065	0.001
7–9	3.530^{b}±0.121	3.794^{a}±0.134	3.548^{b}±0.100	3.652^{ab}±0.108	3.611^{ab}±0.053	0.01
10–12	4.070±0.118	4.117±0.138	4.189±0.020	4.141±0.113	4.126±0.056	NS
3–12	3.590^{b}±0.111	3.851^{a}±0.117	3.716^{ab}±0.007	3.758^{ab}±0.079	3.673^{b}±0.045	0.01
Number of dead birds						
3–12	1.0	2.0	0.0	1.0	0.0	ND
Apparent nutrient digestibility, %						
DM	70.9±1.06	68.4±1.21	70.3±1.33	70.1±1.94	70.3±2.07	NS
CP	89.3^{ab}±0.88	86.4^{b}±1.19	90.0^{a}±1.63	87.9^{ab}±2.08	89.0^{ab}±1.70	0.05
EE	88.1±0.69	87.2±1.29	87.9±1.76	87.7±1.45	88.1±1.13	NS
CF	13.4±0.43	13.0±0.50	13.5±0.85	13.1±0.54	13.3±0.58	NS
OM	80.3±0.73	81.2±4.41	79.8±2.49	79.3±1.66	80.1±1.79	NS

$^{a, b, c}$ Means within a row not sharing a common a superscript differ significantly P≤. 05; NS, not significant; ND, not done.

Table 3 Blood pH, PCV and Hgb before, during and after exposure of slow-growing chicks to heat stress and supplementation of ascorbic acid and different levels of betaine (Mean± SD)

Criteria	Control	Heat stress treatment			Effect of Sampling time
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g/kg	
Blood pH					
Before exposure	7.49±0.015	7.49±0.016	7.49±0.019	7.48±0.014	7.48±0.015	7.49^{c}±0.028
During exposure	7.49^{c}±0.018	7.78 ^{a}±0.027	7.70^{b}±0.018	7.73^{b}±0.038	7.71^{b}±0.026	7.68^{a}±0.095
After exposure	7.49^{b}±0.015	7.59 ^{a}±0.014	7.57^{a}±0.022	7.58^{a}±0.022	7.56^{a}±0.033	7.56^{b}±0.038
PCV						
Before exposure	33.6±0.18	33.6±0.34	33.5±0.38	33.6±0.97	33.5±0.64	33.6^{a}±0.63
During exposure	33.5^{a}±0.96	26.2^{d}±0.74	28.6^{b}±0.74	27.2^{c}±0.95	28.7^{b}±0.74	28.7^{c}±2.46
After exposure	33.6^{a}±1.07	28.3^{d}±0.65	31.0 ^{b}±0.36	28.7^{d}±0.40	29.9^{c}±0.75	30.3^{b}±1.91
Hgb						
Before exposure	8.96±0.083	8.96±0.076	8.95±0.040	8.97±0.064	8.95±0.076	8.96^{a}±0.077
During exposure	8.97 ^{a}±0.046	6.53^{c}±0.182	6.78^{b}±0.122	6.52^{c}±0.056	6.81^{b}±0.071	7.12^{c}±0.867
After exposure	8.97^{a}±0.101	7.51^{c}±0.062	7.78^{b}±0.159	7.71 ^{b}±0.098	7.72^{b}±0.101	7.94^{b}±0.513

$^{a, b, c, d}$ Means within a row for treatments or column for mean effect not sharing a common superscript differ significantly P≤0.05.

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 Hgb 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 Hgb showed the opposite trend over time.

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.

Responses of SRBC's and lymphoid organs

Table (4) shows the impact of different treatments on SRBC's and lymphoid organs. 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.

Rectal temperature

Table (5) shows the influence of different treatments and sampling time on RT. The RT increased ($P<0.05$) due to HS in all groups during all times. However, AA led to a complete relief ($P<0.05$) of negative effect of HS only before heat exposure.

Both doses of Bet was less effective ($P<0.05$) than AA for relief of RT from HS before and during HS. After HS, only 1 g of Bet was the most effective agent ($P<0.05$) for relief RT from HS. 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 of age 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 decreased ($P<0.05$) after HS from 5 wk of age on, then stabilized between

Table 4 Biochemical constituents of blood, response to SRBC's and secondary lymph organs of slow growth chicks exposed to heat stress and supplemented with ascorbic acid and different levels of betaine (Mean± SD)

Criteria	Control (+)	Heat stress treatment			P value
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g/kg	
Blood constituents					
Plasma glucose	226.0^{a}±4.17	213.5^{c}±4.72	223.4^{a}±4.07	217.8^{bc}±4.37	222.0^{ab}±4.47	0.001
Plasma triglyceride	72.4^{d}±1.07	80.2^{a}±1.46	75.8^{c}±2.04	78.5^{b}±1.73	75.8^{c}±1.13	0.003
Serum total protein	4.76^{a}±0.087	4.29^{d}±0.094	4.57^{b}±0.074	4.46^{c}±0.12	4.59^{b}±0.088	0.001
Serum Calcium	7.04^{d}±0.098	7.29^{a}±0.084	7.15^{bc}±0.056	7.20^{b}±0.053	7.10^{cd}±0.055	0.001
Post-injection responses to SRBCs at						
3-days	5.00±0.76	4.50±0.53	5.13±0.83	4.88±0.83	5.25±0.71	NS
6-days	7.25^{ab}±0.89	6.25^{c}±0.71	7.25^{ab}±0.71	6.50^{bc}±0.76	7.50^{a}±0.53	0.006
9-days	4.75^{a}±0.88	3.50^{b}±0.76	4.75^{a}±0.72	3.75^{b}±0.71	4.88^{a}±0.83	0.001
Secondary lymph organs,%						
B. of Fabricius,%	0.220±0.059	0.221±0.064	0.232±0.065	0.209±0.041	0.211±0.069	NS
Thymus,%	0.455±0.064	0.430±0.044	0.450±0.083	0.436±0.087	0.439±0.096	NS
Spleen,%	0.263±0.032	0.227±0.039	0.247±0.041	0.228±0.045	0.237±0.047	0.001

[a, b, c, d] Means within a row not sharing a common a superscript differ significantly P≤.05; NS, not significant.

Table 5 Rectal temperature before, during and after exposure to heat stress and supplementation of ascorbic acid and different levels of betaine (Mean± SD)

Week of age	Control	Heat stress treatment			Age of chicks effect
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g/kg	
Before heat exposure					
3	$40.3^{b}\pm0.17$	$40.4^{a}\pm0.14$	$40.2^{c}\pm0.16$	$40.4^{b}\pm0.19$	$40.4^{b}\pm0.17$	$40.4^{X}\pm0.18$
5	$40.3^{c}\pm0.15$	$40.5^{a}\pm0.19$	$40.3^{c}\pm0.23$	$40.4^{b}\pm0.22$	$40.4^{b}\pm0.17$	$40.4^{X}\pm0.20$
7	$40.3^{c}\pm0.17$	$40.5^{a}\pm0.17$	$40.3^{c}\pm0.17$	$40.4^{b}\pm0.21$	$40.4^{b}\pm0.18$	$40.5^{W}\pm0.20$
9	$40.4^{b}\pm0.11$	$40.6^{a}\pm0.10$	$40.4^{b}\pm0.14$	$40.5^{a}\pm0.11$	$40.4^{b}\pm0.12$	$40.4^{X}\pm0.12$
12	$40.3^{c}\pm0.12$	$40.5^{a}\pm0.10$	$40.4^{b}\pm0.12$	$40.5^{a}\pm0.11$	$40.4^{b}\pm0.12$	$40.4^{X}\pm0.14$
Main effect	$40.3^{C}\pm0.15$	$40.5^{A}\pm0.16$	$40.3^{C}\pm0.18$	$40.4^{B}\pm0.18$	$40.4^{B}\pm0.16$	—
During heat exposure					
3	$40.3^{c}\pm0.17$	$43.6^{a}\pm0.73$	$42.5^{b}\pm0.29$	$42.5^{b}\pm0.25$	$42.6^{b}\pm0.20$	$42.3^{W}\pm0.14$
5	$40.4^{e}\pm0.17$	$43.6^{a}\pm0.41$	$41.3^{d}\pm0.83$	$42.6^{b}\pm0.26$	$41.9^{c}\pm0.46$	$42.0^{X}\pm1.18$
7	40.4^{e} 0.18	$43.2^{a}\pm0.46$	$41.6^{de}\pm0.53$	$42.3^{b}\pm0.29$	41.8^{d} 0.43	$41.9^{Y}\pm0.97$
9	$40.5^{d}\pm0.10$	$43.0^{a}\pm0.32$	$41.6^{c}\pm0.38$	$42.4^{b}\pm0.44$	$41.7^{c}\pm0.35$	$41.8^{Y}\pm0.91$
12	$40.4^{d}\pm0.11$	$42.8^{a}\pm0.54$	$41.7^{c}\pm0.39$	$42.3^{b}\pm0.44$	$41.7^{c}\pm0.38$	$41.8^{Y}\pm0.90$
Main effect	$40.4^{D}\pm0.15$	$43.2^{A}\pm0.60$	$41.7^{C}\pm0.66$	$42.4^{B}\pm0.36$	$41.9^{C}\pm0.50$	—
After heat exposure					
3	$40.4^{c}\pm0.13$	$41.7^{a}\pm0.49$	$40.7^{b}\pm0.20$	$40.6^{bc}\pm0.27$	$40.5^{c}\pm0.21$	$40.7^{X}\pm0.54$
5	$40.5^{d}\pm0.19$	$41.6^{a}\pm0.36$	$40.9^{b}\pm0.70$	$40.6^{c}\pm0.26$	$40.6^{cd}\pm0.27$	$40.8^{W}\pm0.58$
7	$40.5^{b}\pm0.14$	$41.5^{a}\pm0.41$	$40.5^{b}\pm0.20$	$40.5^{b}\pm0.21$	$40.5^{b}\pm0.23$	$40.7^{X}\pm0.49$
9	$40.5^{c}\pm0.10$	$41.2^{a}\pm0.54$	$40.5^{c}\pm0.13$	$40.6^{bc}\pm0.24$	$40.5^{c}\pm0.16$	$40.7^{X}\pm0.40$
12	$40.4^{b}\pm0.10$	$40.6^{a}\pm0.13$	$40.4^{b}\pm0.13$	$40.5^{ab}\pm0.14$	$40.4^{b}\pm0.15$	$40.5^{Y}\pm0.15$
Main effect	$40.4^{D}\pm0.14$	$41.3^{A}\pm0.57$	$40.6^{B}\pm0.40$	$40.6^{B}\pm0.23$	$40.5^{C}\pm0.22$	—

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

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

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 HS was small, however, increased during and after HS 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.

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

Table 6 Respiration rate before during and after exposure to heat stress and supplementation of ascorbic acid and different levels of betaine (Mean± SD)

Week of age	Control	Heat stress treatment			Age of chicks effect*
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g/kg	
Before heat exposure**					
3	55.9±1.5	56.5±1.3	56.5±1.3	56.2±1.1	56.0±1.1	56.2^{W}±1.2
5	54.4 ^{c}±1.2	56.0 ^{a}±1.3	54.6^{c}±1.5	55.3^{bc}±1.8	54.9^{bc}±1.9	55.1^{X}±1.6
7	54.4^{c}±1.4	56.2^{a}±1.2	54.3 ^{c}±1.7	55.5^{b}±1.6	54.8^{bc}±1.7	55.0^{X}±1.7
9	54.0^{b}±1.3	56.0^{a}±1.2	54.3^{b}±1.4	55.0 ^{ab}±1.4	54.1^{b}±1.4	54.7^{Y}±1.5
12	53.8^{c}±1.5	56.0^{a}±1.4	53.8^{c}±1.7	55.5^{ab}±1.5	54.2^{bc}±1.4	54.6^{Y}±1.8
Main effect	54.5^{C}±1.5	56.1^{A}±1.3	54.7^{C}±1.8	55.5^{B}±1.5	54.8^{C}±1.6	—
During heat exposure**					
3	55.9^{d}±1.1	82.4^{a}±2.7	63.5^{c}±2.3	71.2^{b}±3.5	61.9^{c}±4.9	71.1^{W}±10.8
5	55.2^{d}±1.1	80.2^{a}±4.6	65.4^{c}±3.1	69.8^{b}±3.7	65.1^{c}±3.9	67.1^{X}±8.8
7	54.7^{d}±1.2	77.9^{a}±4.8	64.3^{c}±2.8	69.9^{b}±3.5	64.5^{c}±2.9	66.2^{Y}±8.3
9	54.9^{d}±1.1	75.2^{a}±5.3	64.1^{c}±3.1	67.2^{b}±3.4	63.7^{c}±2.8	65.9^{Y}±7.4
12	55.1^{d}±1.2	76.0^{a}±4.2	64.1^{c}±3.5	71.6^{b}±3.5	62.9^{c}±2.9	65.0^{Z}±8.0
Main effect	55.1^{E}±1.2	78.3^{A}±5.2	68.0^{C}±7.8	69.9^{B}±3.8	63.9^{D}±2.3	—
After heat exposure**					
3	55.7^{d}±1.2	60.9^{a}±4.2	60.9^{a}±4.3	57.7^{b}±2.6	56.3^{c}±1.7	58.3^{W}±3.8
5	55.2^{d}±1.0	56.9^{a}±1.5	56.1^{bc}±1.5	56.8^{ab}±2.0	55.9^{cd}±1.8	56.2^{X}±1.7
7	54.9^{d}±1.0	56.7^{a}±1.5	54.9^{d}±1.6	55.8^{bc}±1.7	55.5^{cd}±1.4	56.2^{X}±1.6
9	54.8^{d}±1.1	57.2^{a}±1.8	55.3^{c}±1.5	56.4^{b}±1.8	55.3^{c}±1.4	55.8 ^{XY}±1.8
12	55.1^{d}±1.0	57.5^{a}±1.7	55.6^{cd}±1.6	56.9^{b}±1.7	56.0^{c}±1.2	55.6^{Y}±1.7
Main effect	55.2^{D}±1.1	57.8^{A}±2.8	56.6^{B}±3.2	56.8^{B}±2.0	55.8^{C}±1.5	—

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

^{WXYZ} Means within a column (main effect of chicks age) not sharing a common a 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 a superscript differ significantly P≤.05, based on LSD test.

compared to the other treatment groups during all times, and maximized during HS time while minimized before HS 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 0.5 g of Bet on relief of RR from HS especially before and during HS 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 of age. After HS, the RR decreased ($P<0.05$) after 3 wk of age then stabilized between 5 and 9 wk of age 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.

Obviously, 1 g of Bet 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 was 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 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 approached 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 ($P=0.06$) and 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, provided that 1 g of Bet was more efficient.

Discussion

The Bet in corn and soybean meal were reported to be below the detectable level (Chendrimada et al. 2002), and was not detected in corn-soybean meal diet (Waldroup and Fritts 2005). On the other hand, data related to AA in animal feedstuffs are rare. Although poultry can synthesis AA from glucose, 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 was recently documented, 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

Table 7 Carcass characteristics, chemical composition and physical criteria of meat of slow-growing chicks exposed to heat stress and supplemented with ascorbic acid and different levels of betaine (Mean± SD)

Criteria	Control	Heat stress treatment			P value
			Betaine		
	(-)	+AA	0.5 g/kg	1.0 g/kg	
Carcass criteria					
Dressing, %	70.8^{a}±1.77	68.0^{b}±2.41	70.0^{ab}±3.20	68.2^{b}±3.10	70.8^{a}±2.41	0.001
Abdominal fat, %	0.864±0.046	0.781±0.091	0.815±0.42	0.885±0.121	0.822±0.076	NS
Liver, %	2.45^{a}±0.168	2.10^{c}±0.186	2.36^{ab}±0.14	2.26^{b}±.301	2.35^{ab}±0.156	0.001
Gizzard,%	2.42±0.27	2.42±0.22	2.39±0.16	2.43±0.14	2.44±0.22	0.001
Heart,%	0.48±0.017	0.41±0.056	0.49±0.049	0.45±0.078	0.46±0.057	0.001
Giblets,%	5.35^{a}±0.381	4.93^{b}±0.376	5.24^{a}±0.273	5.15^{a}±0.395	5.24^{a}±0.372	0.001
Pancreas,%	0.190±0.044	0.167±0.019	0.177±0.035	0.167±0.022	0.187±0.022	0.09
Chemical composition of meat on a dry matter basis, %						
DM	24.1^{b}±0.30	25.8^{a}±0.23	24.2^{b}±0.22	24.5^{b}±0.19	24.2^{b}±0.11	0.01
CP	75.8±1.12	75.0±0.24	75.4±0.61	75.1±1.12	75.6±1.17	NS
EE	16.4±0.49	15.3±1.20	16.2±1.76	15.5±1.40	15.5±1.01	NS
Ash	5.00±0.34	5.10±0.40	5.00±0.65	5.00±0.41	5.10±0.203	0.08
Physical parameters of meat						
PH	6.63±0.18	6.59±0.15	6.71±0.09	6.54±0.06	6.62±0.104	NS
Color	0.248±0.025	0.238±0.026	0.234±0.023	0.248±0.021	0.243±0.009	NS
Tenderness	2.36±0.066	2.32±0.047	2.38±0.074	2.31±0.029	2.42±0.031	0.06
WHC	5.66^{a}±0.053	5.43^{b}±0.073	5.61^{ab}±0.131	5.54^{ab}±0.097	5.75^{a}±0.128	0.007

[a, b, c] Means within a row not sharing a common a superscript differ significantly p≤. 0.05, NS, not significant.

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, moisture, tenderness and WHC of meat. Furthermore, physiological traits were correlated with increasing RR and RR e.g. Correlation between RR during HS vs. pH was during (r=0.77; P=0.0001) and was after HS (r=0.67; P=0.0003); Hgb during was (r=−0.70; P=0.0001) and was after HS (r=−0.71; P=0.0001), PCV was during (r=−0.75; P=0.0001) and was after HS (r=−0.73; P=0.0001). The negative effect of HS was for serum glucose (−5.5%), serum total protein (−9.7%), plasma triglycerides (10.8%) and serum Ca (3.6%). Also, correlation between RT during and after HS was (r=−0.51; P=0.009; r=−0.45; P==0.02) for glucose, (r=−0.67; P=0.0003 and r=−0.64; P=0.0006) for total protein, (r=0.58; P=0.002 and r=0.50; P=0.01) for triglycerides, and (r=0.59; P=0.002 and r=0.75; P=0.0001) for Ca. Similar correlation was shown between the aforementioned criteria and RR, too. RT and RR were also correlated only during (r=0.74; P=0.0001 and r=0.63; P=0.0008) and after HS (r=0.57; P=0.003 and r=0.41; P=0.04).

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 test) 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 (Kettunen et al. 2001; Remus et al. 2004) compared to the well-known positive effect of AA (Gous and Morriss 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 1 g 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 Morriss 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 and 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 efficacy 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 Hgb (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 Morriss 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 on (Table 5). There was also an increase in serum total protein and Hgb (Table 4), suggesting an improvement in humoral immunity. The improvements in humoral immune 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, 2006; Gous and Morriss 2005).

Relative weight of dressed carcass, liver and giblets was resorted 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 resort 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 of AA or 1 of 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.

References

Al-Ghamdi, Zahraa, H., 2008. Effects of Commutative heat stress on immunoresponses in broiler chickens reared in closed system. *International Journal of Poultry Science* **7**, 64–968.

AOAC. 1995. Official methods of Analysis, 15th edn, Association of Official Analytical Chemists Washington, DC, USA.

Armstrong, W. D. and Carr, C. W. 1964. Physiological Chemistry Laboratory Direction, 3rd Bursus Publishing Co. Minneapolis, Minnesota, USA.

Attia, Y. A., 2003. Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. *Archiv of Animal Nutrition* **66**, 39–48. doi:10.1080/0003942031000086635

Attia, Y. A., Hassan, R. A., Shehatta, M. H. and Abd El-Hady, S. B., 2005. Growth, carcass quality and blood serum constituents of slow growth chicks as affected by betaine additions to diets containing 2. Different levels of methionine, *International Journal of Poultry Science*, **11**, 856–865.

Attia, Y. A., Bohmer, Barbara M. and Roth-Maier, Dora A., 2006. Responses of broiler chicks raised under constant relatively high ambient temperature to enzymes, amino acid supplementations, or diet density, *Archiv Für Geflügelkunde*, **70**, 80–91.

Chendrimada, T. P., Neto, M. G., Pesti, G. M., Davis A. J. and Bakalli, R. H. I., 2002. Determination of the betaine content of feed ingredients using high-performance liquid chromatography, *Journal of the Science of Food and Agriculture*, **82**, 1556–1563. doi:10.1002/jsfa.1214

Daghir, N. J., 2008. Poultry production in hot climates, 2nd edn. Ed. N. J. Daghir, CAB International.

Eilers, R. J., 1967. Notification of final adoption of an international method and standard solution for hemoglobinometry specifications for preparation of a standard solution. *American Journal of Clinical Pathology*, **47**: 212–214.

Esteve-Garcia, E. and Mack, S., 2000. The effect of DL-methionine and betaine on growth performance and carcass characteristics in broilers, *Animal Feed Science and Technology*, **87**, 85–93. doi:10.1016/S0377-8401(00)00174-7

Garcia N. M.; Pesti, G. M. and Bakalli, R. I., 2000. Influence of dietary protein level on the broiler chickens response to methionine and betaine supplements, *Poultry Science*, **79**, 1478–1484.

Gous, R. M. and Morriss, T.R., 2005. Nutritional interventions in alleviating the effects of high temperatures in broiler Production, *World's Poultry Science Journal*, **61**, 463–475. doi:10.1079/WPS200568

Graham, H., 2002. Betaine-Combating heat stress in poultry, *Afma Matrix*, December, **15**, 16–17.

Hassan, R.A., Attia, Y. A. and El-Ganzory, E. H., 2005. Growth, carcass quality, and blood serum constituents of slow growth chicks as affected by betaine additions to diets containing 1. Different levels of choline, *International Journal of Poultry Science*, **4**, 840–850.

Kai, O.H., Nagase, N., Ishikawa, M., Suzuki, K. and Sato, K., 1988. Effects propylthiouracial PTU on the immunological status of the chickens, *Development comparative Immunology*, **12**, 145. doi:10.1016/0145-305X(88)90032-8

Kettunen, H., Tiihonen, K., Peuranen, S., Saarinen, M.T. and Remus, J. C., 2001. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks, *Comparative Biochemistry and Physiology*, **A 130**, 759–769. doi:10.1016/S1095-6433(01)00410-X

Kutlu, H. R., 2001. Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature, *Archive Für Tierernahrung*, **55**: 127–139.

Lin, H., Du, R. and Zhang, Z. Y., 2000. The peroxidation in tissues of heat-stressed broilers, *Asian-Australian Journal of Animal Science*, **13**, 1373–1376.

Lin, H., Buyse, J., Sheng, Q. K., Xie, Y. M. and Song, J. L., 2003. Effects of ascorbic supplementation on the immune function and laying performance of heat-stressed laying hens, *Journal Feed Agriculture and Environment*, **1**, 103–107.

Lin, H., Jiao, H. C., Buyse, J. and Decuypere, E., 2006. Strategies for preventing heat stress in poultry, *World's Poultry Science Journal*, **62**, 71–85. doi:10.1079/WPS200585

Mahmoud, K. Z., Edens, E. W., Elsen, E. J. and Havenstein, G. B., 2004. Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers Gallus gallus domesticus subjected to cyclic heat stress, *Comparative Biochemical Physiology*, **B 137**, 35–42. doi:10.1016/j.cbpc.2003.09.013

Mashaly, M. M., Hendricks, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O. and Patterson, P. H., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens, *Poultry Science*, **83**, 889–894.

Mujahid, A., Yoshik, Y., Akiba, Y. and Toyomizu, M., 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress, *Poultry Science*, **84**, 307–314.

National Research Cauncil NRC, 1994. Nutrient Requirements of Poultry, 9th revised Edition, National Academy Press. Washington DC., USA.

Pardue, S. I., Thaxton, J. P. and Brake, J. T., 1985. Influence of supplemental ascorbic acid on broiler performance following exposure to high temperature, *Poultry Science*, **64**, 1334–1338.

Puthpongsiriporn, U., Scheideler, S. E., Sell, J. L. and Beck, M. M., 2001. Effects of various E and C supplementation on performance, in Vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress, *Poultry Science*, **80**, 1190–1200.

Remus, J. C., Pierson, E. E. M. and Hruby, M., 2004. The evaluation of betaine and enzymes in coccidian challenged broilers, XXII Poultry Congress, Istanbul, Turkey 8–13 June, 2004.

Sahin, K., Sahin, N., Onderci, I., Gursu, M. F. and Cikim, G., 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and serum metabolites of broiler chickens, Biological Trace Elements Research, 89, 53–64. doi:10.1385/BTER:89:1:53

Sahin, K., Sahin, N., Onderci, I., Gursu, M. F. and Kucuk, G., 2003. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail, Journal of Nutrition, 133, 1882–1886.

SAS Institute 1990 SAS-User's Guide, Statistics, Version 6, 4th edn. SAS Institute Inc., Cary, NC., USA.

Swain, B. K. and Johri, T. S., 2000. Effect of supplemental methionine, betaine and their combinations on the performance and immune response of broilers, *British Poultry Science*, *41*, 83–88. doi:10.1080/00071660086457

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, Animal Clinical Biochemistry, 6, 24.

Türker, M., Alp, M. and Kocabacli, N., 2004. Performance of broiler chicks fed on reduced methionine diets supplemented with betaine, XXII Poultry Congress, Istanbul, Turkey 8–13 June, 2004.

Virtanen, E. and Rosi, L., 1995. Effects of betaine on methionine requirement of broilers under various environmental condition, pp. 88–92 in proceeding of the Australian Poultry Science, Symposium, Sydney, Australia.

Waldroup, P.W. and Fritts, C. A., 2005. Evaluation of separate and combined effects of choline and betaine in diets for male broilers, *International Journal of Poultry Science*, **4**, 442–448.

Wang, Y. Z., Xu, Z. R. and Feng, G., 2004. The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks, *Animal Feed Science and Technology*, **116**, 151–159. doi:10.1016/j.anifeedsci.2004.05.003

Whitehead, C. C. and Keller, T., 2003. An update on ascorbic acid in poultry, *World's Poultry Science Journal*, **59**, 161–184. doi:10.1079/WPS20030010

Yahav, S., Shinder, D., Tanny, J. and Cohen, S., 2005. Sensible heat loss, the broiler's paradox, World's Poultry Science Journal, 61,463–475. doi:10.1079/WPS200568