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ORIGINAL ARTICLE

Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress

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Summary

Four hundred and twenty, 21-day-old slow-growing chicks were divided randomly into seven treatments, each containing five replicates. Each replicate was kept in a 1 × 1-m floor pen. One treatment was kept under thermo-neutral conditions in a semi-open house and fed a corn–soybean meal diet (positive control). The other six groups were kept under chronic heat stress (CHS) at 38 °C and 60% RH for 4 h from 12:00 to 16:00 pm for three successive days per week. Chicks in CHS treatments were fed a corn-soybean meal diet without (negative control) or with increasing metabolizable energy (ME) level by oil supplementation alone, or also with increasing some essential amino acids (EAA) such as methionine (Met), methionine and lysine (Met+Lys) or methionine, lysine and arginine (Met+Lys+Arg) or supplemented with 250 mg of ascorbic acid (AA)/kg.

CHS impaired ($p < 0.05$) growth performance, increased plasma triglycerides and total serum Ca while decreasing ($p < 0.05$) plasma glucose and total serum protein. Meanwhile 250 mg AA/kg diet or an increasing ME without or with some EAA partially alleviated ($p < 0.0001$) the negative effect of CHS on growth while increasing ($p < 0.05$) feed intake and improving ($p < 0.05$) feed:gain ratio (F:G) and crude protein (CP) digestibility ($p < 0.05$). AA or increasing ME with or without EAA increased ($p < 0.05$) percentage dressing, liver and giblets to those of the positive control. AA or increasing ME with or without EAA partially alleviated the negative effect of CHS on blood pH, packed cell volume (PCV), haemoglobin (Hgb), total serum protein and total Ca, plasma glucose and triglyceride, rectal temperature and respiration rate.

Increasing ME level improved chickens' tolerance to CHS without a significant difference from those supplemented with AA. However, increasing Met, Lys and Arg concentration did not improve performance over that recorded with increasing ME level alone. Under CHS, 250 mg AA/kg diet or increasing ME level by addition of 3% vegetable oil could be an useful approach to improve productive and physiological traits of slow-growing chicks, which may be applicable also to fast-growing one.

Introduction

A major challenge facing the poultry industry in the tropics and the subtropics is the high temperature, which negatively affects performance and physiological traits of chickens (Ahmad et al., 2008; Daghir, 2008). The effect of heat stress (HS) on the productive performance of fast-growing chickens has been extensively investigated; however, little attention has been given to slow-growing chickens. However, they contribute 25–30% of the rural poultry production system and thus agriculture development in the tropic and subtropical areas (Daghir, 2008; Attia et al., 2009).

Several nutritional strategies were suggested to relieve the negative effects of HS, e.g. Vitamin C and adjusting dietary levels of metabolizable energy (ME) and protein or amino acids (Sahin and Kucuk, 2001; Ahmad et al., 2008; Daghir, 2008). ME intake is the most important nutrient that limits bird performance in hot climates (Attia et al., 2006; Lin et al., 2006; Daghir, 2008). Feed intake decreases during HS and resulted in decreasing nutrient and ME intake (NRC, 1994; Daghir, 2008). An effective method of increasing ME intake is to include dietary fat/oil due to their beneficial effects (Aggoor et al., 2000).

The requirements for protein and EAA supplementation are independent of environmental temperature so HS does not affect bird performance as long as the protein requirement is met (Daghir, 2008). Thus, protein/amino acid levels need to be adjusted with increasing ambient temperature to decrease heat production associated with their metabolism and help chickens to cope with HS (Attia et al., 2006; Lin et al., 2006). Both protein synthesis and breakdown are affected by CHS (Lin et al., 2006), and protein synthesis is more affected than protein breakdown, leading to reduce protein deposition that cannot be restored by high dietary protein (Temim et al., 2000). Furthermore, growth rate and meat yield of fast-growing chickens are suppressed by a high level of dietary protein (Cahaner et al., 1995), and this effect was dependent on the genotype, showing a nutritional by genotype interaction. Attia et al. (2006) indicated that increasing diet density by ME, Met, Arg and vitamin and mineral supplementation improved broiler performance exposed to heat stress, while Met or Arg alone had no effect. However, adding fat and Lys was shown to improve broiler performance in hot weather (McNaughton and Reece, 1984).

This work aims to investigate the effect of increasing ME level with or without an increase of Met, Lys or Arg compared with AA on productive and physiological traits of slow-growing chickens exposed to CHS to understand whether or not increasing Met, Lys and Arg over increasing ME level is essential under CHS.

Materials and methods

Experimental design, chicks and diets

Four hundred and twenty, 21-day-old slow-growing chicks (El-Salam strain, a white feathers crossbred) *gallus gallus* F. domestica were randomly distributed, keeping equal initial body weight in straight run experimental design among seven treatment groups. Each group contained five replicates with 12 chickens each, 6 males and 6 females. Each replicate was kept in a floor pen (1×1 m) furnished with rice hulls as a litter. Corn–soybean meal diets (Table 1) were fed during the growing period (21–70 days of age) and the finishing period (71–84 days of age).

One treatment group was kept under thermoneutral conditions in a semi-open house with

Table 1 Composition and calculated analyses of the diets fed during growing period (21–70 days of age) and finishing period (71–84 days of age)

Ingredients (g/kg)	Growing	Growing	Finishing	Finishing
Yellow corn	630.5	630.5	733.0	733.0
Soybean meal (44% CP)	330.0	330.0	230.0	230.0
Dicalcium phosphate	20.0	20.0	18.0	18.0
NaCl	3.0	3.0	3.0	3.0
Limestone	9.0	9.0	8.0	8.0
Vit. Min. mixture (premix)*	3.0	3.0	3.0	3.0
Vegetable oil	3.0	33.0	3.5	33.5
Methionine	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5
Total	1000	1030	1000	1030
Calculated and analytical composition				
ME† (kcal/kg)	2881	3053	3005	3174
CP‡ (%)	20.0	19.42	16.50	16.0
Ca† (%)	0.82	0.80	0.81	0.79
Available P† (%)	0.51	0.44	0.46	0.45
Methionine† (%)	0.42	0.41	0.37	0.36
Total sulfur amino acids† (%)	0.75	0.73	0.66	0.64
Lysine† (%)	1.09	1.06	0.85	0.83
Arginine† (%)	1.46	1.42	1.18	1.15
Therionine† (%)	0.88	0.85	0.73	0.71
Tryptophan† (%)	0.28	0.27	0.21	0.20

*Supplied per kg of diet: vitamin A 3.6 mg retinol; vitamin D_3 50 μg cholecalciferol; vitamin E. 10 mg D-alpha-tocopherol; vitamin K_3 2 mg; vitamin B_1 1 mg; vitamin B_2 4 mg; vitamin B_6 1.5 mg; Pantothenic acid 10 mg; vitamin B_{12} 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 1mg; Se 0.1 mg; Mn 55 mg; ethoxyqain 3000 mg.
†Calculated values were according to National Research Council (NRC) (1994) text book values for feedstuffs.
‡Analysed value.

temperature during the experimental period ranging from 25 to 11.6 °C during April, 41.3 to 22.2 °C during May and 29.5 to 18.8 °C during June. The corresponding relative humidity ranged from 95.5% to 33.1%, 40.2% to 11.9% and 95.9% to 38.3% respectively (positive control). Six groups were kept under chronic HS (38 °C and 60% RH for 4 h from 12.00 to 16.00 pm) for three successive days weekly. After the heat episode of each day, birds were kept at a thermo-neutral condition. One group was kept as a negative control; another group was fed a diet supplemented with 250 mg of ascorbic acid/kg diet [a heat-stabilized product produced by Hoffmann-La Roche (Grenzarcherstrausse, Basel, Switzerland)]. The third, fourth, fifth and the sixth groups were fed a diet supplemented with a 3% mixture (1:1:1) of vegetable oils (sunflower, cottonseed and soybean) to increase the ME content of the diet (~170 kcal/kg diet, Table 1). The high ME groups (third, fourth fifth and the sixth groups) was fed the diets without or with 0.08% DL-methionine or 0.08% DL-methionine and 0.12% L-lysine–HCl (78%) or 0.08% DL-methionine, 0.12% L-lysine–HCl (78%), and 0.15% L-arginine. Degussa-AG, Feed Additives, Division D-60287 Frankfurt am Main, Germany donated amino acids.

Feed in mash form and water were provided *ad libitum.* Chickens were vaccinated against New Castle disease using the water-soluble vaccine Hatchner (B_1) at the seventh day of age and with Lasota at 18 and 28 days of age and Losta was repeated every month thereafter. Chickens were vaccinated against Gumboro disease at the 14th and 23rd days of age.

Data collection

All birds were individually weighed (g) every week and simultaneously feed intake (FI) and F:G were recorded on a replicate basis. Mortality rate was recorded daily and used for correction of feed intake/chick/day. At the end of the experiment (84 days of age), 10 birds (5 males and 5 females) per group were slaughtered for evaluation of carcass traits and inner organs. Chemical analyses for CP, lipid, crude fibre (CF), and crude ash (CA) were performed according to AOAC (1995) in skinless-boneless pooled samples (50:50; w/w) of breast plus thigh meat. Meat quality measurements such as meat tenderness and water holding capacity (WHC), meat colour intensity and pH value were determined as outlined by (Attia et al., 2009).

Sheep red blood cells (SRBCs) were used as an antigen test to quantitatively analyses of humoral immune competence. Ten chicks from each treatment group were immunized i.v. via a wing vein with 1 ml of a 10% SRBCs suspension in sterile saline. The chicks were injected just before the heat regimen 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 haemagglutination technique (Kai et al., 1988). Antibody titre values were expressed as $\log^2$ of the highest serum dilution giving total agglutination.

Biweekly rectal temperature (RT) was monitored using a thermo-code electric gauge with an accuracy of 0.1 °C. The respiration rate (RR) was measured by counting the breaths/min by observing abdominal movement for one minute. Blood pH, haemoglobin (Hgb) and packed cell volume (PCV) were determined during the fifth week of age. The measurements for RT, RR, blood pH, Hgb and PCV were taken on 10 birds/treatment. Heparinized blood samples (~3 ml) were taken from the brachial vein to determine pH values using a digital electric pH meter immediately after collection of samples. Hgb concentration was detected as g/dl by the cyanomethemoglobin procedure (Eilers, 1967). Heparinized blood was used for determination of PCV using Wintrobe haematocrit tubes. Blood samples were centrifuged for 20 min at 2200 *g* and PCV values were obtained by reading the PCV on the graduated haematocrit tube.

Ten blood samples per treatment were collected randomly at 12 weeks of age with or without heparin to obtain blood plasma and serum respectively. Plasma and serum were obtained by centrifugation of blood at 2200 *g* for 20 min, and stored at −18 °C for further analyses. Total serum protein (g/dl) was measured by the Biuret method as described by Armstrong and Carr (1964). Plasma glucose concentration (mg/dl) was determined by the method of Trinder (1969). Plasma triglycerides (mg/dl) were determined using Sigma Diagnostics (Sigma Chemical, St. Louis, MO, USA), procedure No. 336. Total serum Ca (mg/dl) was determined by a colorimetric method using available commercial kits (SCLAVO Inc., Wayn, NJ, USA).

At the end of the experiment (12 weeks of age), 10 males from each treatment were housed in separate metabolic cages for 5 days. Birds were given the experimental diets for 3 days as a preliminary period followed by 5 days as a main experimental period, in which quantities of feed intake and excreta (total tract) were determined. The proximate analyses of feed and dried excreta were performed according to AOAC (1995). The apparent digestibility of dry matter (DM), CP, ether extract (EE), CF, and organic matter (OM) was calculated according to Attia et al. (2006).

Statistical evaluation

Data were statistically analysed using one-way ANOVA of SAS® (SAS, 1994) (SAS Institute, Cary, NC, USA) using the following model: ($Y_{ik} = \mu + D_i + e_{ik}$), where Y is a single observation, μ is general mean, D is the effect of treatment (i = A, B, C, D, E, F) and e_{ik} is the random error. Factorial model was used for data of rectal temperature and respiration rate in which the treatments, sampling time and their interactions were the main effects. Mean differences were tested at ($p < 0.05$) using the Student–Newman–Keuls test for main effects and LSD for interaction effects. Chi-squared test was used to test differences for mortality rate.

Results

Productive performance

BWG ($p < 0.0001$) and FI ($p < 0.05$) were decreased while F:G was impaired ($p < 0.02$) due to exposure of chicks to CHS. AA or increasing ME with or without increasing EAA significantly increased BWG and FI and improved F: G of chickens compared with the negative control. However, the impact of treatments was not significant on FI during 21–42 days of age and F:G during 43–63 and 64–84 days of age. AA were the most effective tool for alleviating the negative effect of CHS on BWG compared with increasing the ME level without or with Met, Met+Lys and Met+Lys+Arg during 21–84 days of age. Increasing ME concentration was the most effective for improving F:G in the entire experimental period although not significantly different from other supplementations (Table 2).

Increasing the dietary concentration of Met, Met+Lys and Met+Lys+Arg had no further enhancing effect over that of increasing ME level alone on the BWG, F:G and FI during the most of the experimental period. There were only six chicks that dead during the experimental period (21–84 days of age), which corresponded to 1.4% of the experimental population. This is within a permissible range; however, the highest number was from the unsupplemented CHS group.

Digestibility coefficients

There was no significant effect of CHS and dietary manipulation on apparent digestibility of DM, OM,

Table 2 Productive performance and number of dead birds of slow-growing chicks as affected by chronic heat stress and addition of ascorbic acid or increasing energy concentration without or with some essential amino acid supplementation

		Heat stress treatments							
				Energy groups					
Age	Control (+)	(–)	+ AA	ME	+ Methionine	+ Methionine + lysine	+ Methionine + lysine + arginine	SEM	p-value
Initial body weight (g/bird) and BWG (g/bird)/period									
2 BW (g)	126.3	125.4	126.5	123.1	123.7	123.8	123.8	1.21	NS
BWG 21–42 days	380.3^{a}	321.3^{d}	356.5^{c}	357.7^{c}	358.0^{c}	361.0^{c}	367.1^{b}	1.44	0.0001
BWG 43–63 days	359.8^{a}	314.3^{c}	361.8^{a}	343.3^{b}	349.5^{b}	343.3^{b}	343.1^{b}	2.93	0.0001
BWG 64–84 days	398.6^{a}	377.2^{b}	393.4^{a}	397.7^{a}	394.0^{a}	388.7^{a}	392.5^{a}	3.23	0.0003
BWG 21–84 days	1138.7^{a}	1012.8^{e}	1111.7^{b}	1098.7^{cd}	1101.5^{c}	1093.0^{d}	1102.7^{c}	2.31	0.0001
FI (g/bird)/period (days)									
21–42	1171.8	1122.4	1171.6	1117.4	1143.6	1151.6	1148.6	13.66	NS
43–63	1328.1^{a}	1254.9^{c}	1343.3^{a}	1308.7^{b}	1313.3^{ab}	1329.3^{a}	1318.3^{ab}	16.35	0.04
64–84	1561.1^{a}	1487.9^{b}	1581.3^{a}	1547.4^{ab}	1551.4^{ab}	1576.3^{a}	1551.0^{ab}	17.26	0.04
21–84	4061.0^{ab}	3865.2^{b}	4096.2^{a}	3973.5^{ab}	4008.3^{ab}	4057.2^{ab}	4017.9^{ab}	44.10	0.05
F:G (kg/kg)/period (days)									
21–42	3.17^{c}	3.63^{a}	3.40^{b}	3.23^{c}	3.30^{bc}	3.30^{bc}	3.23^{c}	0.0372	0.0001
43–63	3.56	3.82	3.57	3.67	3.62	3.72	3.69	0.0592	NS
64–84	4.10	4.14	4.22	4.08	4.13	4.26	4.14	0.0535	NS
21–84	3.57^{b}	3.82^{a}	3.68^{ab}	3.62^{b}	3.64^{ab}	3.71^{ab}	3.64^{ab}	0.0436	0.02
Mortality									
Number of dead birds	(1/60)	(2/60)	(0/60)	(1/60)	(1/60)	(0/60)	(1/60)	0.38	NS*

Means within a row not sharing a common a superscript differ significantly; NS, not significant; SEM, standard error of the means.
*Chi-square test was used to test differences among mortality rate.

EE and CF. Addition of AA and increasing ME with Met+Lys+Arg significantly increased digestibility of protein compared with the negative control. Nonetheless, increasing ME level with only Met or Met+Lys did not yield a further improvement in digestibility of protein compared with increasing ME level alone. However, CHS did not negatively affect digestibility of protein compared with the positive control (Table 3).

Lymphoid organs and humoral immune response to SRBCs

Chronic heat stress significantly decreased the spleen percentage compared with the positive control, while addition of AA or increasing ME concentration with or without addition of EAA relieved this negative effect. CHS and dietary manipulation had no effect on percentage thymus and Fabricii bursa. The humoral immune response to SRBCs was not affected by CHS and dietary manipulations at 3-, 6- and 9-days post-immunization (Table 3).

Carcass characteristics, inner organs and meat quality

Chronic heat stress significantly decreased percentage dressing, liver, giblets, and meat moisture compared with the positive control. Tenderness, WHC, pH and colour intensity were not significantly affected by CHS and AA supplementation. Percent pancreas, heart, gizzard, proventricules, abdominal fat and percentage CP, lipid and ash of meat were not significantly affected by different treatments. AA addition or increasing ME concentration with or without some EAA-induced similar effects and alleviated the negative effects of CHS on percentage liver and giblets. Increasing ME with or without EAA significantly improved meat tenderness, while decreasing meat WHC compared with the positive control, negative control and the AA groups (Table 4).

Blood pH, PCV and Hgb and metabolic profile

Chronic heat stress significantly increased the pH value of the blood, plasma triglycerides and total serum Ca while significantly decreasing PCV, Hgb, total serum protein and plasma glucose compared with the positive control. Blood pH, plasma triglycerides and total serum Ca significantly decreased, whereas PCV, Hgb, total serum protein and plasma glucose significantly increased compared with the negative control because of AA supplementation. Increasing ME concentration showed a similar effect to those observed with AA for alleviating the negative effect of CHS on total serum protein and Ca. However, increasing the ME level had stronger effect than AA for blood pH, meanwhile the opposite trend was observed for blood PCV and plasma glucose (Table 5).

Table 3 Apparent nutrient digestibility, lymphoid organs and responses to SRBCs of slow-growing chicks as affected by chronic heat stress and addition of ascorbic acid or increasing energy concentration without or with some essential amino acid supplementation

		Heat stress treatments							
				Energy groups					
Criteria	Control (+)	(–)	+ AA	ME	+ Methionine	+ Methionine + lysine	+ Methionine + lysine + arginine	SEM	p-value
Apparent nutrient digestibility (%)									
DM	70.90	68.85	70.75	67.70	70.30	70.03	69.90	0.989	NS
OM	80.33	81.23	79.83	79.95	80.00	80.10	79.98	1.24	NS
CP	89.30^{ab}	86.35^{b}	90.00^{a}	89.20^{ab}	89.35^{ab}	89.25^{ab}	89.63^{a}	0.721	0.03
EE	88.08	87.18	87.90	88.25	88.70	88.50	88.00	0.662	NS
CF	13.43	12.95	13.50	13.88	13.95	13.75	13.50	0.321	NS
Lymphoid organs (%)									
Spleen	0.262^{a}	0.220^{b}	0.246^{a}	0.236^{a}	0.238^{a}	0.242^{a}	0.232^{a}	0.0104	0.0001
Thymus	0.453	0.427	0.448	0.440	0.459	0.438	0.446	0.0195	NS
Bursa of fabricius	0.220	0.222	0.232	0.222	0.216	0.208	0.229	0.0388	NS
Humeral immune responses to SRBC's test									
24 days	5.00	4.50	5.13	4.75	4.88	5.00	5.00	0.266	NS
27 days	7.25	6.25	7.25	6.88	6.88	7.00	7.00	0.281	NS
30 days	4.75	3.50	4.75	4.13	4.25	4.63	4.75	0.300	NS

Means within a row not sharing a common a superscript differ significantly; NS, not significant; SEM, standard error of the means.

Table 4 Carcass characteristics (%), chemical composition (%) and physical characteristics of meat of slow- growing chicks as affected by chronic heat stress and addition of ascorbic acid or increasing energy concentration without or with some essential amino acid supplementation

		Heat stress treatments							
				Energy groups					
Criteria	Control (+)	(–)	+ AA	ME	+Methionine	+Methionine + lysine	+ Methionine + lysine +arginine	SEM	p-value
Carcass characteristics and inner organs (%)									
Dressing	70.3^{a}	67.6^{b}	69.5^{ab}	67.9^{b}	69.0^{ab}	68.6^{ab}	68.9^{ab}	0.44	0.0001
Liver	2.44^{a}	2.07^{b}	2.35^{a}	2.35^{a}	2.54^{a}	2.38^{a}	2.53^{a}	0.051	0.0001
Pancreas	0.189	0.166	0.177	0.179	0.190	0.176	0.176	0.0074	NS
Heart	0.447	0.432	0.478	0.442	0.440	0.451	0.454	0.0092	NS
Gizzard	2.40	2.41	2.39	2.35	2.45	2.50	2.42	0.059	NS
Giblets	5.32^{a}	4.90^{b}	5.22^{a}	5.15^{a}	5.42^{a}	5.33^{a}	5.40^{a}	0.068	0.0001
Proventriulus	0.369	0.365	0.377	0. 377	0.377	0.390	0.388	0.0089	NS
Abdominal fat	0.219	0.220	0.232	0.221	0.214	0.206	0.224	0.0187	NS
Carcass composition (%)									
Moisture	75.9^{a}	74.0^{b}	76.2^{a}	75.9^{a}	75.2^{ab}	75.6^{ab}	75.2^{ab}	0.37	0.02
Protein	18.3	19.4	18.3	18.4	18.9	18.5	18.8	0.33	NS
Lipids	3.96	3.96	3.94	4.33	4.47	4.49	4.53	0.130	NS
Ash	1.21	1.32	1.20	1.19	1.24	1.28	1.20	0.054	NS
Physical characteristics of meat									
Tenderness (cm^2)	2.40^{b}	2.34^{b}	2.40^{b}	2.56^{a}	2.58^{a}	2.54^{a}	2.61^{a}	0.036	0.002
WHC (cm^2)	5.70^{a}	5.80^{a}	5.65^{a}	5.39^{b}	5.44^{b}	5.36^{b}	5.41^{b}	0.044	0.0001
pH value	6.67	6.68	6.78	6.55	6.68	6.64	6.70	0.062	NS
Color intensity	0.245	0.242	0.240	0.279	0.308	0.310	0.272	0.015	NS

Means within a row not sharing a common a superscript differ significantly; NS, not significant; SEM, standard error of the means.

Table 5 Hematological and biochemical constituents of blood of slow- growing chicks as affected by chronic heat stress and addition of ascorbic acid or increasing energy without or with some essential amino acid supplementation

		Heat stress treatments							
				Energy groups					
Criteria	Control (+)	(–)	+ AA	ME	+Methionine	+ Methionine + lysine	+ Methionine + lysine + arginine	SEM	p-value
Hematological constituents of blood									
Blood, %	2.72	2.73	2.71	2.70	2.70	2.73	2.71	0.144	NS
pH	7.54^{d}	7.68 ^{a}	7.63^{b}	7.59^{c}	7.58 ^{c}	7.59 ^{c}	7.60^{bc}	0.011	0.0001
PCV, %	33.81^{a}	29.51^{d}	31.16^{b}	30.12^{c}	30.24 ^{c}	30.38 ^{c}	30.49^{c}	0.161	0.0001
Hgb, g/dl	9.00^{a}	7.73 ^{c}	7.89^{b}	7.79^{bc}	7.79^{bc}	7.79^{bc}	7.82^{bc}	0.028	0.0001
Biochemical constituents of blood									
Total serum protein, g/dl	4.82^{a}	4.35^{c}	4.61^{b}	4.63^{b}	4.58^{b}	4.67 ^{b}	4.60^{b}	0.031	0.0001
Plasma glucose, mg/dl	230.1^{a}	219.4^{b}	227.6^{a}	219.8^{b}	222.2^{b}	220.6^{b}	222.4^{b}	1.41	0.0001
Plasma triglycerides, mg/dl	72.8^{c}	80.5^{a}	76.1^{b}	75.6^{bc}	75.6^{bc}	75.5^{bc}	75.5^{bc}	0.72	0.0001
Total serum calcium, mg/dl	7.11^{b}	7.36^{a}	7.20^{b}	7.14^{b}	7.16^{b}	7.14^{b}	7.17 ^{b}	0.035	0.0001

Means within a row not sharing a common a superscript differ significantly; NS, not significant; SEM, standard error of the means.

Rectal temperature and respiration rate

There was a significant increase in rectal temperature and respiration rate due to CHS. AA or high ME diets partially relief the negative effects of CHS on RT and RR. However, AA was more effective for RT than high ME diet fed with or without some EAA. In contrary, high ME diet supplemented with the three amino acids was more effective for decreasing RR than AA although not significantly

Table 6 Rectal temperature (°C) and respiration rate (breath/min) during heat exposure course as affected by chronic heat stress and addition of ascorbic acid or increasing energy concentration without or with some essential amino acid supplementation

		Heat stress treatments								
				Energy groups						
Age of chicks (wk)	Control (+)	(–)	+ AA	ME	+ Methionine	+ Methionine + lysine	+ Methionine + lysine + arginine	Chicks' age effect*	SEM	p-value
Rectal temperature (°C)										
4	40.4^{e}	43.6^{a}	42.6^{bc}	42.6^{bc}	42.6^{bc}	42.7^{b}	42.5^{cd}	42.4^{V}	0.785	0.0001
6	40.5^{e}	43.4^{ab}	41.8^{d}	42.4^{cd}	42.4^{cd}	42.5^{cd}	42.4^{cd}	42.2^{W}		
8	40.5^{e}	43.0^{b}	41.7^{d}	42.2^{cd}	42.6^{cd}	42.4^{cd}	42.2^{cd}	42.0^{X}		
10	40.5^{e}	42.8^{bc}	41.6^{d}	41.6^{d}	42.0^{cd}	42.1^{cd}	42.0^{cd}	41.9^{Y}		
12	40.3^{e}	42.6^{bc}	41.7^{d}	41.5^{d}	41.7^{d}	41.6^{d}	41.6^{d}	41.6^{Z}		
Main effect	40.5^{E}	43.1^{A}	41.9^{c}	42.2^{B}	42.3^{B}	42.3^{B}	42.2^{c}	0.0001	0.026	0.0001
Respiration rate (breath/min)										
4	55.8^{f}	82.4^{a}	63.5^{de}	63.1^{de}	63.5^{de}	63.1^{de}	62.0^{e}	65.7^{V}	0.545	0.0001
6	55.1^{f}	80.1^{ab}	65.3^{de}	64.4^{de}	66.5^{d}	65.1^{de}	63.0^{e}	64.7^{W}		
8	55.7^{f}	77.8^{b}	64.2^{de}	63.9^{de}	65.4^{de}	64.4^{de}	64.1^{de}	64.9^{W}		
10	54.8^{f}	75.1^{bc}	64.0^{de}	64.0^{de}	64.9^{de}	64.1^{de}	63.4^{de}	64.3^{W}		
12	55.1^{f}	76.0^{bc}	62.0^{e}	62.4^{e}	65.9^{de}	64.6^{de}	64.0^{de}	64.6^{W}		
Main effect	55.1^{E}	77.8^{A}	64.1^{C}	63.7^{CD}	65.3^{B}	64.0^{C}	63.2^{D}	0.0001	0.182	0.0001

Means within a row (main effect of treatment) not sharing a common a superscript differ significantly, based on SNK test;
Means within a column (main effect of chicks age) not sharing a common a superscript differ significantly, based on SNK test;
Means within treatments within weeks (interaction effect) within each time of exposure not sharing a common a superscript differ significantly, based on LSD test.
*SEM for the effect of chick's age on rectal temperature = 0.030 and that for respiration rate = 0.206.
SEM, standard error of the means.

different from increasing ME level alone. Met addition over high ME diet significantly increased RT and RR compared with increasing ME level alone. The same trend was observed in only RT when Met+Lys was supplemented; however, Met+Lys decreased RR compared with increasing Met level alone. Meanwhile, Met+Lys+Arg addition induced no beneficial over increasing ME level alone for the RT and RR (Table 6).

Age of chicks had significant effects on RT and RR showing that RT decreased with increasing age of chicks and reached the minimum value at the end of the experiment (12 week of age). On the contrary, the RR was not significantly changed after 6 week of age. This indicates chickens had better control of the RR than RT.

There was a significant interaction between age of chicks and treatments on RT and RR. The changes over time in RT and RR were greater in CHS groups than the thermo-neutral groups. There were no changes in the RT and RR over time in the positive control group. In the negative control group, there were gradual decreases in RT and RR with increasing chicks' age. Differences between the positive and the negative controls were significant at all tested age.

There was a significant decrease in RT and RR due to AA addition compared with the negative control when corresponding ages were compared; however, the values were still significantly higher than those recorded by the positive control at all tested ages. Feeding high ME diet yielded similar effects on RT and RR to those observed in the AA supplemented-group while supplementing with EAA did not differ from high ME alone.

Discussion

Effect of heat stress

A huge body of data can be located on the effect of HS on fast-growing chickens, however, little attention has been given to slow-growing one, which contributes to ~30% of poultry production in developing countries and this was one of the main objectives of the present work. Exposure to CHS decreased BWG by 15.5%, 12.6%, 5.4%, and 11% during 21–42, 43–63, 64–84 and 21–84 days of age, respectively, compared with the positive control. In addition, from 43 days of age on, the FI of the CHS group significantly decreased by 5.5% and 4.7% during 43–63 and 64–84 days of age, respectively, compared with the

positive control. However, the decrease during 21–84 days of age was insignificant (3.7%) compared with the positive control. This could explain a part 33.7% of the decrease in growth of the CHS group. In this regard, Dale and Fuller (1979) indicated that a 63% reduction in growth of fast-growing broilers at high temperature was due to a decrease in FI. According to National Research Council (NRC) (1994), broiler's FI will be depressed by approximately 1.5% for each increase of 1 °C above thermo-neutral. Furthermore, Faria Filho et al. (2006) showed that the direct effect of temperature accounts for 39% of body weight decrease and for 100% of poor F:G of HS broilers.

Slow-growing chickens exposed to CHS had significantly worse F:G of 14.5% and 7.0% during only 21–42 and 21–84 days of age, respectively, compared with the positive control (Table 2). This showed poor F:G during only the early age that affect F:G for the entire period. However, May and Lott (2000) reported that HS did not affect F:G in broiler chickens. The impact of CHS on BWG, FI and F:G decreased with increasing age of chicks. These agreed with those reported by Yahav and Hurwitz (1996) and Yahav and Plavnik (1999) who found that thermal conditioning at an early age resulted in complete growth compensation by the second week of age and exceeded that of the control at 5 or 6 weeks of age.

Chronic heat stress significantly decreased dressing percentage (Table 4) and this might be due to decreasing nutrient availability for growth rate (Table 2). Chronic heat stress significantly decreased percentage spleen, liver, dressing, moisture of meat, PCV (12.7%), Hgb (14.1%), total serum protein (9.8%) and plasma glucose (4.7%) compared with the positive control. The decrease in PCV and Hgb may elucidate the increase in RT and RR of the CHS group (Table 6). Meanwhile, CHS significantly increased the pH value of blood (1.9%), and plasma triglyceride (10.7%) and total serum Ca (3.5%). Similarly, Shoukry (2001) revealed that high temperature had no effects on carcass weight, dressing percentage, gizzard weight and heart weight of broiler chickens. However, Temim et al. (2000) reported that exposure of broiler chickens to 32 °C for 2 weeks during 4–6 weeks of age increased abdominal fat, whereas decreasing absolute and relative weight of breast meat compared with the control kept at 22° C. Similarly, Sandercock et al. (2001) and Nassiri Moghaddam et al. (2006) reported that HS increased blood pH, a condition referred to as alkalosis associated with increasing panting. In addition, HS (38 ± 1 °C for 3 h at 36 and 37 days of age) caused a significant decrease in haematocrit value from 34.6% to 31%, and total protein and glucose. The increase in blood glucose level may be due to increasing efforts to decrease heat load (Tollba et al., 2004) as a result of increasing glucocorticoid secretion that increased gluocogenesis, and/or intestinal mucosa to uptake hexose (Garriga et al., 2006).

The increases in RT and RR resulted from CHS (Table 6) are in agreement with those reported by Lin et al. (2006) and Al-Ghamdi (2008). Mortality was very low and one could not draw a precise picture, since two birds died from the CHS group.

Effect of ascorbic acid or high-energy diet with or without amino acid addition

Ascorbic acid alleviated the negative effect of CHS on BWG throughout the experimental period and this coincided with increasing FI (Table 2). The positive effect of AA on growth rate was 11%, 15.1%, 4.3% and 9.8% during 21–42, 43–63, 64–84 and 21–84 days of age, respectively, compared with the CHS group. Similarly, Sahin et al. (2003) observed that 250 mg/kg AA preventing HS-related depression in performance of broiler chickens. The mechanism of AA in relieving the negative effect of HS may be due to its suppressive effect on plasma corticosterone (Mahmoud et al., 2004), adrenocorticortropic hormone (Sahin et al., 2003), and increased serum T_3 and T_4 and birds' appetite (Sahin et al., 2002).

Increasing ME level significantly increased BWG of chicks compared with the CHS group, however was less effective than AA, especially during 43–63 and 21–84 days of age. However, both agents had similar potentiality during 21–42 and 64–84 days of age. The improved growth rate of high ME diet groups was coincided with a significant increase in FI during only 43–63 days of age. Similarly, Mateos et al. (1982), Attia et al. (2006) and Daghir (2008) demonstrated that increasing diet density by fat addition increased BWG and improved F:G of broilers exposed to high temperature.

Increasing Met, Met+Lys or Met+Lys+Arg over increasing ME level did not yield further improvement in BWG of chicks throughout the most of the experimental period except for 21–42 days of age, when addition of the three EAA was found to be the most potent compared with other dietary manipulations including AA. This indicated higher Agr requirements for growth during 21–42 days of age compared with the other ages. In agreement with

the lack of response to Met addition, Balnave et al. (1999) concluded that the growth response to DL-Met at 32 °C was optimized at a low (1.03) dietary Arg:Lys ratio and the response was affected by source of Met. In general, the lack of response to Met, Lys and Arg supplementations on growth for the entire period is in agreement with those reported by Attia et al. (2006) who found that increasing Met, Lys and Arg over ME level did not improve growth performance of broilers. Furthermore, Lin et al. (2006) concluded that the suppression of growth from HS reduces the absolute requirements for amino acids. The ideal protein balance under HS remains unclear, as altered digestion, absorption of amino acids, protein synthesis and the higher protein catabolism and gluconeogenesis was recorded in HS chickens (Lin et al., 2005; Daghir, 2008).

There was no growth response to increasing Lys level over Met during the most of the experimental periods (Table 2). These results are similar to those reported by March and Biely (1972) who reported that high temperature (31 °C) did not affect the metabolic requirements for Lys. The ideal sulphur amino acid:Lys ratio used herein was 0.68 during 21–42 days of age and 0.78 during 43–84 days of age and in the range 0.75–0.78 suggested by National Research Council (NRC) (1994) and Fisher (2002). In this regard, Veldkamp et al. (2005) reported that increasing Met, Lys and threonine did not improve growth of turkeys subjected to HS. It was observed that during 21–84 days of age increasing Lys over Met and ME significantly decreased BWG compared with Met supplementation and which corrected by increasing Arg level (Table 2). Scott et al. (1982) reported also that a small increase in Lys level can cause a marked elevation of kidney arginase activity and increased Arg degradation, thus causing growth depression in chicks because of the Lys–Arg antagonism. Increasing the Arg level above Met and Lys significantly improved chick growth during only 21–42 compared with the other ME groups, during 21–84 days of age compared with a Lys supplemented-diet, and overcame the negative effect of Lys for the whole period (Table 2). Similarly, broilers' sustainment to HS improved because of increasing Arg uptakes from the gut (Balnave and Brake, 2002, 2005). However, Mendes et al. (1997) observed that increasing the Arg:Lys ratio up to 1.3 had no significant effect on growth performance of broilers. The lack of consistency of Arg supplementation noticed in the literature might be due to the Arg:Lys ratio, and the impact of dietary NaCl on the Arg:Lys ratio (Balnave and Brake, 2002). In addition, Fisher (2002) reported that Arg requirements for chicken growth are different under a variety of environmental conditions and need further investigation.

Ascorbic acid relieved the negative effect of CHS on F:G of slow-growing chickens partially during 21–42 days of age and completely during 21–84 days of age with improvements of 6.34% and 3.66% respectively. Sahin and Kucuk (2001) and Naseem et al. (2005) reported similar results. However, Ladmakhi et al. (1997) showed that AA up to 500 ppm had no effect on F:G of broilers maintained in the cyclic temperature (24–35 °C) during 28–42 days of age.

Increasing ME level by oil addition significantly improved F:G by 11.0 and 5.2% during 21–42 and 21–84 days of age compared with the negative control and resulted in diminishing of the negative effect of CHS on F:G (Table 2). Similarly, Abdel-Fattah et al. (2005), Attia et al. (2006) and Swatson (2006) reported that chicks supplemented with corn oil had better F:G and performance index compared with control group during the summer.

Increasing the EAA concentration over increasing ME level did not improve F:G compared with increasing ME level alone. Nonetheless, high-ME diet with or without addition of Met+Lys+Arg yield significantly better F:G compared with AA supplemented-group only during 21–42 days of age. However, this effect was not significantly different from the other ME groups. This means that increasing ME level by 170 kcal ME by 3% oil addition is adequate for improving growth performance of slow-growing chickens exposed to CHS. Similarly, Swatson (2006) and Attia et al. (2006) found that increasing Met level or Arg did not affect F:G of broiler chicks. However, Corzo et al. (2003) showed that increasing the Lys level seems to be essential to accommodate depressed FI and improve the F:G of broilers.

Supplementing AA or increasing ME level with supplementation of Met+Lys+Arg improved digestibility coefficients of protein, which may explain the improved growth performance of these groups. In partial agreement with our results, Sahin and Kucuk (2001) and Sahin et al. (2003) found that 200–250 ppm AA improved apparent digestibility of DM, OM, CP and EE of Japanese quails exposed to 33 °C. However, Attia et al. (2006) cited that increasing dietary Met, Arg and diet density did not affect N-retention.

Supplementing AA or increasing ME level with or without EAA supplementation significantly, increased percentage liver and giblets thus the difference from the positive control was diminished

(Table 4). Meanwhile, AA and increasing Met alone over ME level improved dressing percentage significantly to the level of the positive control, showing that higher Met requirements for carcass traits. Similarly, Sahin and Kucuk (2001), Sahin et al. (2003) and Attia et al. (2009) reported that AA addition to HS-Japanese quail and slow-growing chickens improved carcass traits. In addition, Attia et al. (2005) indicated that Met addition to a corn and soybean meal diet for slow-growing chicken improved breast meat yield.

Ascorbic acid significantly decreased blood pH (6.5%), plasma triglyceride (5.5%) and total serum Ca level (2.2%), while increasing PCV (5.6%), Hgb (2.10%), total serum protein (5.7%) and plasma glucose (3.73%) compared with the CHS group (Table 5). These metabolic changes indicate the antistress effect of AA (Lin et al., 2006). On the contrary, the increase in blood PCV, Hgb, and glucose coincided with the decrease in RT and RR (Table 6). Furthermore, the increase in total serum protein showed additional beneficial effects of AA (Table 5). The increase in PCV because of AA addition is in agreement with the results of Curca (1993) who found that chickens given AA diet had a higher haematocrit value and Hgb. The improvements in blood PCV and Hgb concentration observed herein might be due to the positive known effect of AA on improving iron absorption (Attia et al., 2009). In partial agreement with the present results, Sahin et al. (2003) noted that 250 mg AA/kg diet decreased serum glucose of broiler chickens reared at 32 °C whereas total serum protein was increased in Japanese quails kept at 34 °C for 8 h.

Increasing ME level had similar effects to AA for alleviating the negative effect of CHS on total serum protein and Ca. However, increasing ME level had more beneficial effects than AA for blood pH may be due to the acidity of fatty acids. Meanwhile, the opposite trend was observed for blood PCV and plasma glucose. Increasing EAA concentration over ME level did not yield further improvements in haematological and biochemical constituents compared with increasing ME level alone (Table 5), indicating that increasing ME alone was adequate for revealing the negative effects of CHS on physiological traits. Similarly, Attia et al. (2005, 2006) noticed that the Met level or Arg:Lys ratio at high temperatures did not affect blood parameters of broilers.

In conclusion, CHS decreased productive performance and impaired physiological traits of slow-growing chickens, whereas addition of 250 mg AA/kg diet or increasing ME level by addition of 3% vegetable oil similarly relief the negative effects of CHS. Meanwhile, increasing Met, Lys and Arg levels over ME level did not improve productive performance or physiological response over that recorded with increasing ME level alone, showing that increasing ME level under CHS is adequate for slow-growing chickens, which may be applicable to fast-growing one.

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