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Broiler tolerance to heat stress at various dietary protein/energy levels

Hitzetoleranz von Broilern bei Fütterung von Rationen mit variierenden Protein- und Energiegehalten

Youssef A. Attia[1*] and **Saber S. Hassan**[2]

[1] Arid Land Agriculture Department, Faculty of Meteorology, Environment, and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

[2] Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Egypt

*Correspondence: yaattia@kau.edu.sa

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Introduction

Heat stress represents a challenge to poultry production worldwide, and has a negative influence on performance, product quality and the health of chickens (Daghir, 2008; Syafwan et al., 2011). Diet adjustments, vitamin and mineral supplementation and improved housing conditions have been suggested as useful tools to overcome negative effects of heat stress (Attia et al., 2011; Syafwan et al., 2011; Suganya et al., 2015). Nonetheless, nutrition manipulation under heat-stress conditions may have a greater impact than feed additives due to a decrease in feed intake and thus in nutrient intake during heat stress (Veldkamp et al., 2000). Heat stress decreases the consumption of feed, nutrients and metabolisable energy (ME) (NRC, 1994; Daghir, 2008; Attia et al., 2011). It has been suggested to include dietary fat/oil in broiler diets to increase ME intake and decrease the heat increment (Aggoor et al., 2000; Attia et al., 2003; Ghazalah et al., 2008).

Protein and energy are the main elements responsible for metabolism, enzymes, hormones, tissue growth and egg formation. Thus, the growth of chickens during heat stress may be limited by the availability of protein/amino acids and/or energy (Attia et al., 2006; Lin et al., 2006; Daghir, 2008). Decreasing protein and/or energy can reduce the performance of broilers under heat stress to a degree that may not be compensated by feed-additive supplementations (Attia et al., 2011). On the other hand, protein metabolism is associated with greater heat increments and thus the energy needs for growth and reproduction (Brake et al., 1998). In the literature, the effect of protein and/or energy concentration on broilers exposed to heat stress is contradictory. For example, the requirements for protein and amino acids are constant regardless of the environmental temperature as long as the protein/amino acid requirements are met (Veldkamp et al., 2000; Daghir, 2008; Attia et al., 2006; 2011). Protein metabolism (synthesis and breakdown) is influenced by chronic heat stress (CHS) (Lin et al., 2006), and the synthesis of protein is influenced more than the breakdown of protein, leading to decreased deposition of protein that cannot be restored by increasing the protein concentration in the feed (Temim et al., 2000). On the other hand, the growth and meat yield of broilers are decreased by increasing the protein concentration in their feed (Cahaner et al., 1995). This influence is dependent on the genotype of broilers, showing an interaction between nutrition and genotype. This suggests that adjusting protein/amino acid levels during hot weather may be a useful tool to improve the tolerance of chickens to CHS (Attia et al., 2006; Lin et al., 2006). Nonetheless, Veldkamp et al. (2000) with turkeys and Attia et al. (2011) with chickens indicated that increasing the concentration of essential amino acids such as methionine, lysine, arginine and threonine does not improve broiler performance compared to increasing energy concentration. Increased energy concentration and the addition of lysine and/or fats and/or oils to the feed enhanced the production of broilers in hot climates (McNaughton and Reece, 1984). In addition, Attia et al. (2006) found that increasing ME, methionine, arginine, mineral and vitamin concentration by 10% in broiler diets during CHS increased growth performance, while increasing methionine and arginine alone did not influence broiler production traits. Increasing energy concentration by using fat/oil supplementation increased growth performance of broilers exposed to heat stress (Al-Harthi et al., 2002; Lou et al., 2003; Raju et al., 2004; Ghazalah et al., 2008; Attia et al., 2011). Nonetheless, fat supplementation added to broiler diets under heat-stress conditions did not affect broiler performance (Sinurat and Balnave, 1985). Moreover, decreasing the metabolisable energy concentration during heat exposure has been recommended (Baghel and Pradhan, 1990; Hoffmann et al., 1991). Thus, increasing energy levels by increasing protein levels may be a useful nutritional technique that may help overcome the negative effects of heat stress (Attia et al., 2006; Daghir, 2008; Attia et al., 2011). There is currently a lack of studies on the effect of increasing protein when combined with increasing energy concentration on the performance of broilers exposed to CHS. Thus, this research examines the effect of increasing the dietary protein concentration with or

without increasing the energy levels in order to improve the tolerance of broiler chickens to heat stress and improve their performance traits.

Materials and methods

Chickens, experimental design and diets

A total of 140, 28-day-old male Ross-308 broiler chickens were randomly distributed, keeping equal the initial body weights, in a straight-run completely randomised experimental design among 4 treatment groups. Each treatment consisted of 7 replicates and 5 male chicks per replicate. Each replicate was kept in battery brooders (35×25×30 cm l-w-h). The batteries were placed in a semi-open house setting that was equipped with two air-turn ceiling fans, two wall exhaust fans and three manual gas heaters to keep the temperature constant. The housing was divided into two sections; one was used for the thermoneutral treatment and the other for the heat stress condition. The walls were separated by walls made of wood and there was an adequate distance between each portion. The control in temperature was done manually by watching the thermometer and adjusting the heaters every hour to keep the desired temperature during the heat-stress period.

One treatment group was kept under a thermoneutral condition (28 ± 4°C and 40–60% RH) and was fed on a diet containing 19% CP and 13.2 MJ ME/kg diet on days 28–49 (thermoneutral). The other three groups (2, 3 and 4) were kept for 4 successive days weekly under 36 ± 3°C and 40–60% RH for 6 h daily from 10 a.m. to 4 p.m. Group 2 was kept under CHS and fed on the same diet and was considered a negative control. Group 3 was kept under CHS and fed on a diet containing 22% CP and 13.2 MJ ME/kg. Group 4 was kept under CHS and fed on a diet containing 22% CP and 13.8 MJ ME/kg.

Broiler husbandry

During the pre-experimental period – days 1–28 – the birds were kept under similar managerial and hygienic conditions. The chickens were fed corn-soybean meal feeds in a mash form during days 1–27, as shown in Table 1. The husbandry of the chickens was performed according to the Ross Broiler Management Handbook (http://ar.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Handbook-2014i-EN.pdf). Feed and water were provided *ad libitum*. Their vaccination program was Hitchiner + IB on day 8, avian influenza (H_5N_2) on day 9, Gumboro on day 14 and day 24 and Newcastle disease virus (NDV) via Lasota on days 14, 20 and 30. The chickens were illuminated with a 23:1 light-dark cycle.

Table 1. Ingredients and chemical composition of the experimental diets

Inhaltsstoffe und Nährstoffgehalte der Versuchsrationen

Ingredients, g/kg	Strater diet, 1–27 day of age	Grower-finsher diet,28–49 days of age		
		19% CP, 13.2 MJ ME/kg	22% CP, 13.2 MJ ME/kg	22% CP, 13.8 MJ ME/kg
Yellow corn	550.0	630.0	546.0	510
Soybean meal	320.0	259.0	315.0	321.1
Corn gluten meal	55.0	38.0	60.0	60.0
Limestone	11.8	12.0	12.4	12.4
Dicalcium phosphate	18.0	14.0	14.0	14.0
Vit+Min Premix1	3.0	3.0	3.0	3.0
NaCl	3.0	3.0	3.0	3.0
DL-Methionine	1.6	1.5	0.9	0.9
L-Lysine (HCL)	1.8	2.5	0.7	0.6
Vegetable oils[2]	35.8	37.0	45.0	75.0
Total	1000	1000	1000	1000
Analysed[3] and calculated[4] values (g/kg)				
Dry matter[3]	878	894	882	889
ME MJ/kg[4]	12.9	13.2	13.2	13.8
CP[3]	217	187	218	219
Methionine[4]	5.4	4.8	4.8	4.8
Sulphur amino acids[4]	9.1	8.1	8.4	8.4
Lysine[4]	12.0	11.0	11.0	11.0
SAA/lysine ratio[4]	76	74	76	76
Calcium[4]	9.9	8.8	9.0	9.0
Available phosphorus[4]	4.8	3.9	4.0	4.0
Crude fat[3]	52.9	54.3	61.9	891.
Crude fibre[3]	36.5	33.3	34.5	32.6
Ash[3]	65.1	55.3	55.7	54.3

[1]Vit+Min mixture provides per kg of the diet: vitamin A (retinyl acetate) 24 mg, vitamin E (dl-α-tocopheryl acetate) 20 mg, menadione 2.3 mg, Vitamin D3 (cholecalciferol) 0.05 mg, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, choline chloride 600 mg, vitamin B12 10 µg, vitamin B6 3 mg, thiamine 3 mg, folic acid 1 mg, d–biotin 0.50 mg. Trace mineral (mg per kg of diet): Mn 80 Zn 60, Fe 35, Cu 8, Se 0.60.
[2] A mixture of soybean oil, cotton seed oil and sunflower at 33.33% of each.

Data collection

At 28 and 49 days of age, the broilers were weighed (g), and their feed intake was recorded for the same period. In addition, the feed conversion ratio (FCR), the protein conversion ratio (PCR) – which is equal to the amount of protein consumed (dietary determined CP × feed intake) divided by body weight gain – and the ME conversion ratio (MECR) – which is equal to the amount of ME consumed (dietary calculated ME × feed intake) divided by the body weight gain – were calculated. The survival rate was recorded as the number of live chickens at the end of the experimental period, relative to the initial number of chickens. The European production index (EPI) was calculated according to ATTIA et al. (2012). The cloacal temperature was measured after emptying of the cloaca from excreta by a digital thermometer with a sensitivity of 0.1°C. The respiration rate was done by counting the number of abdominal movements per minute. The measurements were taken once a week, just after exposure to heat stress.

On day 49, one chicken was chosen randomly from each treatment replicate. The 7 chickens per treatment were weighed after being fasted overnight, slaughtered according to the Islamic method, their feathers picked, and the total inedible parts removed. Then the remaining carcass (the dressed weight) was weighed. The proventriculus, gizzard, heart, liver, pancreas, intestines, abdominal fat, spleen, fibrous bursa and thymus were separated and weighed. The relative weight of the carcass and inner organs to the live body weight were calculated.

A meat sample (n = 7 per treatment), represented by 50% of thigh deboned meat and 50% of breast deboned meat of the slaughtered chickens, was collected on day 49. About 200 g of each sample was wrapped and frozen at –18°C until used for chemical analyses. A part of the fresh meat samples was used to determine the physical characteristics of the meat (n = 7 samples per treatment). The method of VOLVOINSKAIA and KELMAN (1962) was used to determine the water-holding capacity (WHC) and tenderness of the meat. Colour intensity as an optical density of the meat and drip and the pH of the meat and

drip were assessed according to HUSANI et al. (1950) and AITKEN et al. (1962), respectively. The chemical analyses of meat such as dry matter, protein, ether extract and ash were determined according to ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, AOAC (2004).

Seven blood samples per treatment were collected on day 49 in unheparinised and heparinised tubes to determine some of the haematological and biochemical constituents. Blood samples were centrifuged at 3000 rpm for 20 minutes, and the plasma and serum were stored at −20°C for further analyses. The blood's haematological characteristics, such as haemoglobin (Hgb) and PCV, were determined according to EILERS (1967), red blood cells (RBCs) were determined according to HEPLER (1966), and the blood MCV, MCH and MCHC were calculated. The white blood cells (WBCs) were measured according to LUCAS and JAMROZ (1961); the phagocyte index (PI) and activity (PI) were determined according to LEIJH et al. (1986), and plasma glucose (TRINDER, 1969), serum total protein (WEICHSELBAUM, 1946), serum albumin (DOUMAS et al., 1977) and serum globulin (COLES, 1974) were determined. In addition, the albumin-to-globulin ratio was calculated. The serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were evaluated according to REITMAN and FRANKEL (1957). Renal function, creatinine and urea were appraised in serum according to BARTLES et al. (1972) and SAMPSON et al. (1980), respectively, and the urea-to-creatinine ratio was calculated. Alkaline phosphatase (ALP) enzymes were gauged according to KIND and KING (1954) and total plasma cholesterol was assessed according to the method of WATSON (1960). The plasma T3 was done as per YOUNG et al. (1975) and cortisone by ELSIA kits (product number, ABIN 5210527; www.antibodies-online.com).

The method by KORACEVIC et al. (2001) and RICHARD et al. (1992) was used to determine the plasma total antioxidant capacity (TAC) and malondialdehyde (MDA), respectively. Glutathione reductase (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were assessed according to MANNERVIK and CARLBERG (1985), CHIU et al. (1976) and MISRA and FRIDOVICH (1972), respectively. Catalase was determined using a Sigma Aldrich kit (Catalase Assay Kit Catalogue Number CAT100, St. Louis, MO 63103, USA).

Serum antibody body titres for NDV and avian influenza (AI) were measured (TAKATSY, 1956; KAI et al., 1988), and infectious bursal disease (IBD) was determined according to COSGROVE (1962). Serum immunoglobulin (IgG, IgM and IgA) was determined using a commercial ELISA kit according to BIANCHI et al. (1995).

Statistical evaluation

An analysis of variance was done using one-way analyses of variance according to SAS® (2009), using the following model: $Y_{ij} = \mu+F_i+e_{ij}$, where Y = the dependent variable, μ = overall mean; F_i = the effect of heat stress treatments and e_{ij} = the random error. The replicate was the experimental unit. All percentages were transformed to log10 to normalise the data distribution before analyses. The mean difference at $P \leq 0.05$ was tested using the Student Newman Keuls test. The survival rate was analysed using the chi-square test.

Results

The effects of heat stress and dietary protein and energy concentration on the growth performance of broilers are presented in Table 2. The results indicate significant negative effects of CHS on body weight gain (BWG), feed and nutrient intake, nutrient-conversion ratio, survival rate and EPI. However, increasing protein concentration with or without increasing energy concentration significantly improved body weight gain, feed, protein and energy intake, utilisation of energy, protein and feeds, survival rate and EPI. on the other hand, increasing protein with increasing energy concentration was more effective for BWG, FCR and EPI than increasing protein alone.

Table 2. Effect of heat stress and increasing dietary protein and energy levels on growth performance of broiler chickens during 28–49 days of age

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf die Wachstumsleistung der Broiler zwischen dem 28. und 49. Lebenstag

Treatments	Body weight gain, g 28–49 day of age	Feed intake, g 28–49 day of age	Protein intake, g	ME intake, MJ	Feed conversion ratio, 28–49 day of age	Protein conversion ratio, g/g	ME conversion ratio, kJ/g	Survival rate, %	European production Index
Thermoneutral	1069^{a}	2074^{a}	388^{c}	27.3^{b}	1.94^{c}	0.363^{c}	25.6^{b}	100^{a}	261^{a}
CHS	844^{c}	1896^{c}	354^{d}	25.0^{c}	2.26^{a}	0.422^{b}	29.8^{a}	97.1^{b}	185^{b}
CHS- HCP	939^{b}	2042^{a}	445^{b}	26.9^{a}	2.17^{ab}	0.474^{a}	28,7^{a}	100^{a}	206^{b}
CHS-HCPME	1029^{a}	2096^{a}	459^{a}	29.0^{a}	2.05^{bc}	0.448^{ab}	28,3^{a}	100^{a}	242^{a}
Statistical analyses									
SEM	25.2	19.4	3.69	0.257	0.054	0.011	0.178	4.75	10.94
P Value	0.001	0.001	0.001	0.001	0.033	0.001	0.033	0.001	0.006

[1]Number of observations were 7 replicates per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = standard error of mean, CHS = chronic heat stress, CHS-HCP = chronic heat stress fed on high protein diet, CHS-HCPME = chronic heat stress fed on high protein and high ME diet.

There was a significant effect from the treatments on proventriculus, liver and intestinal percentage (Table 3), showing that increasing protein with increasing energy concentration significantly reduced proventriculus percentage compared to the other groups, while increasing protein concentration alone significantly increased liver and intestinal percentage, compared to the other groups. There was no significant effect of increasing dietary protein concentration with or without increasing energy concentration on percentage dressing, gizzard, heart, pancreas and abdominal fat.

Table 3. Effect of heat stress and increasing dietary protein and energy levels on carcass characteristics and inner organs at 49 days of age

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf die Schlachtkörpermerkmale der Broiler am 49. Lebenstag

Treatments	Dressing,%	Proventriculus,%	Gizzard,%	Liver, %	Heart,%	Pancreas,%	Intestine,%	Abdominal fat,%
Thermoneutral	71.8	0.300^{a}	1.19	2.08^{b}	0.461	0.185	4.52^{b}	0.995
Heat stress	72.6	0.324^{a}	1.12	2.14^{b}	0.451	0.163	4.22^{b}	1.46
CHS- HCP	72.6	0.308^{a}	1.07	4.47^{a}	0.586	0.196	6.03^{a}	1.54
CHS-HCPME	71.7	0.241^{b}	1.09	2.79^{b}	0.537	0.175	3.37^{b}	1.62
Statistical analyses								
SEM	2.036	0.018	0.066	0.425	0.036	0.014	0.331	0.208
P Value	0.096	0.046	0.692	0.008	0.082	0.494	0.001	0.249

[1]=Number of observations were 7 chickens per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = standard error of mean, CHS = chronic heat stress, CHS-HCP = chronic heat stress fed on high protein diet, CHS-HCPME = chronic heat stress fed on high protein and high ME diet.

There were no significant differences on dressing, proventriculus, gizzard, liver, heart, pancreas, intestinal and abdominal fat (Table 3), as well as chemical (DM, CP and ash) and physical traits (pH, colour as measured by optical density, tenderness and WHC) of meat (Table 4), except for meat lipids, which increased significantly due to CHS treatments compared to the thermoneutral group and further increased due to the high-protein diet alone.

Table 4. Effect of heat stress and increasing dietary protein and energy levels on meat quality traits at 49 days of age

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf die Fleischqualitätsmerkmale der Broiler am 49. Lebenstag

	Chemical composition of meat, %				Physical characteristics of meat			
Treatments	Dry matter	Crude protein	Lipids	Ash	pH	Colour (optical density)	Tenderness cm^2/g	WHC, cm^2/g
Thermoneutral	25.8	19.2	5.63^{c}	0.990	5.96	0.126	8.67	15.8
Heat stress	26.4	19.4	5.88^{b}	0.984	6.13	0.106	8.88	15.5
CHS- HCP	26.1	19.1	6.07^{a}	0.982	5.98	0.118	8.73	15.8
CHS-HCPME	26.0	19.0	5.89^{b}	0.984	6.02	0.128	9.03	15.7
Statistical analyses								
SEM	0.152	0.159	0.044	0.006	0.045	0.007	0.111	0.116
P-value	0.147	0.361	<.0001	0.776	0.072	0.143	0.159	0.141

[1]=Number of observations were 7 samples per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stress fed on high protein and high ME diet, pH = hydrogen power.

Blood-protein metabolites, liver function and renal function are shown in Table 5. Chronic heat stress did not significantly affect protein metabolites or the indices of liver and renal functions, with the exception of a significant increase in AST compared to the other groups. Feeding a high-protein diet significantly increased ALT, compared to feeding a high-protein with high-energy diet, ALT/AST compared to the thermoneutral and CHS groups and uric acid compared to the other groups. Treatments did not affect blood-protein metabolites, urea, creatinine or the urea-creatinine ratio. In addition, high protein with a high-energy diet did not affect the AST ratio, compared to the high-protein diet group and the thermoneutral group, the ALT/AST ratio compared to the other groups and uric acid compared to the thermoneutral and CHS groups.

Table 5. Effect of heat stress and increasing dietary protein and energy levels on protein metabolites and indices of liver and renal functions

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf Metaboliten des Proteinstoffwechsels und auf Indizes der Leber- und Nierenfunktion

	Protein metabolites, g/dl				Liver function indices U/l			Renal function indices			
Treatments[1]	Total protein, g/dl	Albumin, g/dl	Globulin, g/dl	Alb/Glb ratio	ALT, IU	AST, IU	ALT/AST ratio	Uric acid, µg/ml	Urea, mg/dl	Creatinine, g/dl	Urea/creatinine
Thermoneutral	5.67	3.54	2.14	1.70	67.4^{ab}	56.8^{b}	1.19^{b}	2.25^{b}	23.4	0.994	24.3
Heat stress	5.75	3.59	2.16	1.68	65.8^{ab}	60.0^{a}	1.10^{b}	2.44^{b}	24.6	1.15	22.0
CHS- HCP	5.66	3.66	2.02	1.83	68.9^{a}	53.8^{b}	1.28^{a}	2.80^{a}	24.5	1.01	24.6
CHS-HCPME	5.74	3.61	2.10	1.72	65.0^{b}	53.3^{b}	1.22^{ab}	2.47^{b}	25.4	1.06	24.1
Statistical analyses											
SEM	0.041	0.062	0.066	0.078	0.875	1.587	0.019	0.084	0.547	0.057	1.34
P-Value	0.319	0.858	0.723	0.745	0.022	0.007	0.018	0.001	0.161	0.172	0.347

[1]=Number of observations were 7 plasma samples per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stress fed on high protein and high ME diet, Alb/Glb = Albumin/globulin ratio, ALT = Alanine amino transferease, AST = Aspartate amino transferease.

Groups on the chronic heat-stress treatments significantly increased plasma cholesterol and glucose, cloaca temperature and respiration rate compared to the thermoneutral group, but there was no effect on alkaline phosphatase or plasma T_3 (Table 6). In addition, the CHS group had significantly elevated alkaline phosphatase compared to the group on the high-protein with high-energy diet, but did not differ from other groups.

Table 6. Effect of heat stress and increasing dietary protein and energy levels on blood biochemical constituents, cloaca temperature and respiration rate

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf biochemische Blutparameter, die Rektaltemperatur und die Respirationsrate

Treatments[1]	Cholesterol, mg/dl	Alkaline phosphatase, U/l	Glucose mg/dl	T_3, ng/ml	Cortisone, ng/ml	Cloaca temperature,°C	Respiration rate, breath/minute
Thermoneutral	205^{b}	11.5^{ab}	213^{b}	2.21	4.29^{b}	41.6^{b}	43.6^{b}
Heat stress	214^{a}	12.1^{a}	239^{a}	2.28	4.65^{a}	42.6^{a}	76.3^{a}
CHS- HCP	214^{a}	11.0^{ab}	244^{a}	2.29	4.05^{b}	42.5^{a}	76.9^{a}
CHS-HCPME	214^{a}	10.7^{b}	248^{a}	2.26	4.30^{b}	42.3^{a}	68.7^{a}
Statistical analyses							
SEM	1.27	0.264	3.82	0.026	0.087	0.111	3.9
P-Value	0.0002	0.0008	<.0001	0.337	0.004	0.001	0.001

[1]=Number of observations were 7 samples per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stress fed on high protein and high ME diet, T3 = Tri-iodiothreonine

Table 7 shows the influence of different treatments on the haematological parameters of broiler chickens. RBC parameters except for PCV and WBCS characteristics were not significantly affected by different treatments. The PCV was significantly decreased due to the CHS treatment compared to the thermoneutral group, but similarly increased due to being fed on high-protein and high-protein with high-energy diets compared to the thermoneutral and CHS groups.

Table 7. Effect of heat stress and increasing dietary protein and energy levels on red blood cells and white blood cell characteristics

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf Charakteristika der roten und weißen Blutkörperchen

	Red blood parameters						White blood parameters					
Treatments[1]	RBCS,10^6 cell/mm^3	Hgb, g/dL	PCV,%	MCV, fl/cell	MCH, pg	MCHC,%	WBCs,10^3 cell/mm^3	Lymphocyte,%	Monocyte,%	Basophile,%	Eosinophile,%	Heterophile,%
Thermoneutral	1.54	11.1	33.1^{b}	219	73.7	33.5	23.9	43.3	12.5	7.00	8.25	29.0
Heat stress	1.45	10.8	31.9^{c}	226	75.7	34.0	25.0	43.9	12.1	6.75	8.62	28.6
CHS- HCP	1.51	12.2	36.5^{a}	242	80.8	33.3	24.8	45.0	11.5	8.25	8.00	27.5
CHS-HCPME	1.65	11.2	34.0^{a}	207	67.9	32.8	22.7	44.0	11.4	6.87	8.12	29.6
Statistical analyses												
SEM	0.051	0.441	1.09	10.6	3.85	0.809	0.633	0.856	0.492	0.491	0.292	1.114
P-value	0.052	0.118	0.013	0.125	0.142	0.531	0.087	0.612	0.831	0.203	0.660	0.563

[1]=Number of observations were 7 blood samples per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$).
SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stres high protein and high ME diet, WBC`s = White blood cell, HLR = Heterophile/Lymphocyte ratio, RBCs = Red blood cells; Hgb = Haemogobluin, PCV = cell volume, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, HLR = Heterophil/lymphocyte r

Table 8 shows the effect of different treatments on lymphoid organs and immune indices. Spleen percent, PI, HIAI, HIND and HIIBD were not significantly affected by treatments. Chronic heat-stress significantly increased bursa percentage compared to the thermoneutral treatment. In addition, feeding a high-protein with high-energy diet significantly decreased thymus (%) compared to feeding a high-protein diet alone, but there was decreased PA compared to the thermoneutral group.

Table 8. Effect of heat stress and increasing dietary protein and energy levels on the relative weight of spleen, bursa of Fabricius and thymus and antibody titres of broiler chickens

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf die relativen Gewichte von Milz, Bursa Fabritius und Thymus sowie auf die Antikörpertiter

Treatments[1]	Spleen,%	Buras,%	Thymus,%	PA,%	PI,%	HIAI*, log 2	HIND*, log 2	HIIBD*, log 2
Thermoneutral	0.096	0.023^{b}	0.179^{ab}	17.0^{b}	7.97	3.37	4.62	3.75
Heat stress	0.144	0.037^{a}	0.154^{ab}	19.1^{ab}	8.34	3.75	5.00	3.75
CHS- HCP	0.153	0.039^{a}	0.193^{a}	19.0^{ab}	7.65	3.12	4.25	3.87
CHS-HCPME	0.119	0.042^{a}	0.090^{b}	20.0^{a}	8.00	3.00	4.12	3.75
Statistical analyses								
SEM	0.0187	0.0039	0.021	0.594	0.283	0.255	0.221	0.213
P-value	0.242	0.034	0.033	0.023	0.595	0.261	0.189	0.969

[1]=Number of observations were 7 samples per treatment.
* Geometric means
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stress fed on high protein and high ME diet, PA = Phagocyte activity, PI = Phagocyte index, HIND = Newcastle disease, HIIBD = Infection bursa disease; HIAI = Avian Influenza.

Table 9 displays the impact of heat stress and increasing protein and energy levels on antioxidant status and immunoglobulins of broilers. Glutathione reductase, glutathione peroxidase and MAD were not significantly affected by the treatments. SOD was significantly decreased due to exposure to CHS, while none of the different dietary manipulations showed a positive effect. Catalase was significantly increased due to CHS, whereas increasing the protein level with or without energy concentration restored the catalase in the thermoneutral group without significantly affecting the other treatments. Total antioxidant capacity was significantly increased by CHS and increased further due to increasing protein and energy concentration, without there being distinguishable differences between the groups on high protein or high-protein with high-energy diets.

Table 9. Effect of heat stress and increasing dietary protein and energy levels on antioxidant status and immunoglobulins

Einfluss von Hitzestress und erhöhten Protein- sowie Energiegehalten im Futter auf den antioxidativen Status und die Immunglobuline

Treatments[1]	SOD, IU/l	Catalase, nmol/min/ml	Glutathione reductase, IU/l	Glutathione peroxidase, mg/l	TAC, nmol/ml	MDA, µmol/ml	IgA mg/dl	IgM mg/dl	IgG mg/dl
Thermoneutral	259^{a}	34.1^{b}	26.6	43.4	423^{c}	0.874	75.0^{b}	247^{b}	969^{c}
Heat stress	253^{b}	36.3^{a}	27.0	41.6	429^{b}	0.867	80.5^{a}	255^{a}	975^{b}
CHS- HCP	253^{b}	35.1^{ab}	27.1	41.9	437^{a}	0.842	76.8^{b}	249^{b}	983^{a}
CHS-HCPME	252^{b}	35.2^{ab}	26.1	42.0	436^{a}	0.894	77.9^{ab}	247^{b}	979^{ab}
Statistical analyses									
SEM	1.56	0.329	0.293	0.816	1.72	0.018	0.826	2.19	1.57
P-value	0.028	0.001	0.131	0.365	0.0007	0.271	0.001	0.006	<.0001

[1]=Number of observations were 7 per treatment.
Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), SEM = Standard error of mean, CHS = Chronic heat stress, CHS-HCP = Chronic heat stress fed on high protein diet, CHS-HCPME = Chronic heat stress fed on high protein and high ME diet, TAC = Total antioxidant capacity, MAD = Malondialdehyde, IgA = Immunoglobulin A, IgM = Immunoglobulin M, IgG = Immunoglobulin G.

Exposure to CHS significantly increased IgA, IgM and IgG compared to the thermoneutral group. on the other hand, increasing protein concentration with or without elevating energy concentration resulted in a similar restoration of IgA and IgM to the level of the thermoneutral group.

Increasing protein level alone caused a significant decrease in IgA and IgM compared to the CHS group, but increased IgG compared to the other groups, with the exception of the high-protein with high-energy group. on the other hand, increasing concentration of protein with energy decreased only IgM compared to the CHS group, but did not affect IgA and IgG, compared to the CHS and high-protein groups.

Discussion

Effect of chronic heat stress

During days 28–49, exposure to CHS significantly decreased BWG by 21%, feed, protein and energy intake ≈8.6% and impaired FCR, CPCR and MECR by ≈16.5%, compared to the thermoneutral group. CHS also induced 2.9% more mortality and significantly decreased EPI, by 29.1%. Increasing metabolic heat negatively influences feed intake (Al-Harthi et al., 2002; Ghazalah et al., 2008). The decreases in feed, CP and ME intake accounted for 41% of the decrease in growth. We found that feed intake decreased by 1.1% for each 1°C increase in temperature, which is very close to the published results found in the literature, of 1.2%-1.5% (Rose and Michie, 1987; NRC, 1994; Veldkamp et al., 2000). The present results are in agreement with those reported by Faria Filho et al. (2006, 2007), who observed that the impact of temperature on the feed intake of broiler chickens accounted for 39% of their reduction in growth and 100% of their poor feed utilisation. High environmental temperatures stimulate the peripheral thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus. The result is a reduction in the consumption of feed and thus of nutrients available for heat dissipation, hormone synthesis and enzymatic activities.

The decrease in growth and impaired FCR was accompanied by an increase (4.44%) in meat lipid content, showing that under CHS, chickens increased fat deposition instead of tissue growth due to decreased protein synthesis (Cowan and Michie; 1978; Veldkamp et al., 2000). In addition, there was an increase (5.6%) in liver enzyme (AST), showing impaired hepatic cellular permeability. The liver is the site of protein metabolism. These results are similar to those cited by Tollba et al. (2004) and by Attia et al. (2009; 2011).

The metabolic profiles showed negative effects from heat stress; e.g., total cholesterol (+4.4%), alkaline phosphatase (+5.5%), glucose (+12.5%) and cortisone (+8.4%). The cloaca temperature and respiration rates were also increased. The latter was also evident by the panting behaviour of the chickens during the experimentation period, particularly during the CHS period. These results are in line with those reported by Attia et al. (2006; 2011).

The decreased PCV (3.63%) indicates a reduction in RBCs due to loss of RBCs as a result of cell destruction in heat-exposed animals (Attia et al., 2009). Antioxidant enzymes showed different trends due to CHS, as SOD decreased by 2.2% but catalase and TAC increased by 6.3 and 1.6%, respectively. The decrease in SOD indicates a reduction in the ability of broilers to resist the damage of oxygen-free radicals and other stress factors. SOD is an essential defence enzyme that catalyses the reduction of hydrogen peroxides into oxygen and water during CHS, protecting the DNA from damage and cells from reactive-oxygen species (Attia and Kamel, 2012). The increase in catalase in broilers exposed to CHS stress showed that SOD is the first defence mechanism and catalase the second for removing the toxic hydrogen peroxide (Rindler et al., 2013).

Immunoglobulins (humoral immunity IgA, IgM and IgG) and lymphoid organs such as bursa showed different trends due to CHS, significantly increasing by 7.3%, 3.5% 0.5% and 60.9%, respectively, compared to the thermoneutral group. The increased immunoglobulin in the CHS group in comparison to the thermoneutral group indicates a role for humeral immunity (immunoglobulin) in disease protection during CHS. Immunoglobulins such as IgG, IgA and IgM show an essential role in the binding of foreign antigens. The presence of these antibody molecules on a microbial or parasitic surface can cause clumping (agglutination). IgG and IgM also activate the complement system (Tizard, 1998; Ayaz et al., 2008). In addition, bursa produced B cells, which function in the humoral immunity component of the adaptive immune system is secreting antibodies. Furthermore, B cells present antigen that classify as professional antigen-presenting cells and secrete cytokines (Cooper, 2015).

The decrease in PCV and increase in glucose, cholesterol, alkaline phosphatase, cortisone and AST showed the negative effect of CHS on haematological and immunological traits. Similarly, Attia et al. (2009; 2011) found that CHS impairs the haematological and immunological traits of chickens. The increase observed herein in plasma glucose could be attributed to impaired glucose tolerance (Tollba et al., 2004) resulting from increased secretion of glucocorticoids, which increased glycogenesis and/or of the uptake of hexose by the intestinal mucosa (Garriga et al., 2006).

Effect of increasing protein concentration

Feeding a high-protein diet significantly increased BWG by 11.3%, feed and energy intake by 7.7%, protein intake by 25.7%, improved FCR and MECR by 4.0% and 3.5%, respectively and EPI by 11.4% compared to the CHS group, while impairing PCR by 12.3%. These results indicate that increasing the protein concentration from 19% to 22% was beneficial for broilers raised under chronic heat stress. This could be attributed to the increased feed and energy intake, which could account for 68.1% of the improved BWG. Similarly, Faria Filho et al. (2006, 2007) observed that increased protein concentration from 17% or 20% to 23% tended to improve the growth and FCR of broilers reared at 32°C, while decreasing protein utilisation. In addition, Cowan and Michie (1978) observed that turkeys increased protein consumption at high ambient temperatures when fed on a high-protein diet to maintain a constant protein intake. The current results indicate that growth and FCR, PCR and MECR were enhanced due to increasing dietary protein concentration. However, the complete recovery was not achieved because the thermoneutral group had higher growth and better FCR than the high-protein group.

Increasing the dietary protein concentration caused a significant increase in the liver percentage of 109% and the intestinal percentage (42.9%), compared to the CHS group, while decreasing the AST, compared to the CHS group. The impaired protein utilisation of the high-protein group was accompanied by increased meat lipids (3.2%) and uric acid (24.4%), compared to the CHS group. These results are similar to those reported by VELDKAMP et al. (2000), who found that extra protein/amino acids have to be de-aminated and the carbon skeleton used as an energy source, enabling the chickens to deposit more fat. The increase in uric acid reflected the extra N from deamination.

Increasing the dietary protein concentration significantly improved animal welfare, as judged by the decrease in plasma cortisone. Thus, the difference to the thermoneutral group was diminished. The improved animal welfare was accompanied by increasing PCV compared to the CHS and the thermoneutral groups. The increase in TAC indicated that the broilers attempted to resist the damage by oxygen-free radicals and other stress factors (ATTIA et al., 2011). These results indicate that increasing the dietary protein concentration had a positive impact on humeral immunity and bursa percentage (B-lymphocyte), and this was accompanied by an increase in TAC. It is well-known that protein/amino acids, fatty acids and antioxidants are essential components in antibody synthesis (ABAZA, 2002).

Effect of increasing protein with increasing energy concentration

Increasing the protein concentration combined with increasing the energy concentration by increasing the oil supplementation of the diet to 7.5% was more effective for recovering the negative influence of CHS than was increasing the protein concentration alone. However, a complete recovery was not achieved as the thermoneutral group had numerically higher BWG (3.7%) and better FCR (5.7%) and EPI (7.9%) than the high- protein with high-energy group.

Increasing the protein with increasing energy concentration compared to increasing the protein concentration alone significantly increased BWG (by 21.9% vs 11.3%), protein intake (by 29.7% vs 25.7%) and EPI (by 30.8% vs 11.4%). These results indicate that increasing the protein concentration from 19 to 22%, when combined with increasing the energy concentration from 13.18 to 13.81 MJ/kg by adding 3% more oil was more beneficial for broilers raised under CHS than increasing protein concentration alone. This could be attributed to the increased feed, energy and protein intake, which could account for 27.6% and 78.5%, 32.8%, respectively, of the improved growth. The increase in energy/fat intake showed the greatest contribution to the enhancement in the growth of broilers. This could be attributed to the impact of fat/oils on improving the palatability of the diet and the low heat increment of the fat/oil-containing diet compared to protein and carbohydrates (AGGOOR et al., 2000; GHAZALAH et al., 2008; SYAFWAN et al., 2011; SUGANYA et al., 2015). The latter authors showed broilers housed at 29–36°C benefitted from dietary supplementation with 5% poultry fat (GHAZALAH et al., 2008). Fat supplementation resulted in a dietary extra caloric effect due to increasing the ME of other resources in the feed (MATEOS and SELL, 1981). It enhanced nutrient utilisation due to the reduction in feed-passage time and improved the digestibility of organic matter (MATEOS et al., 1982; GHAZALAH et al., 2008). In addition, the increase in dietary energy concentration resulted in a decrease in the use of protein intake for growth (from 95.4% vs. 20.4%), showing an improvement in the protein utilisation for growth, compared to increasing the protein concentration alone. These results are in line with those reported by ATTIA et al. (2006, 2011) and SUGANYA et al. (2015). In accordance with the present results, ME inadequate to sustain the processes of protein synthesis diverts energy and protein away from growth (HURWITZ et al., 1980; VELDKAMP et al., 2000; ATTIA et al., 2003), and extra protein can contribute to the dietary heat increment (BRAKE et al., 1998). In accordance with the present results, MCNAUGHTON and REECE (1984) found that increasing lysine and ME significantly increased the productive performance of broilers in hot climates. In addition, AL-HARTHI et al. (2002), LOU et al. (2003), RAJU et al. (2004) and GHAZALAH et al. (2008) found that increasing the ME contents of the diet by 5% fat supplementation increased growth and improved the FCR and production indices.

The decrease in proventriculus and intestinal percentage and liver due to increasing the protein and energy concentration compared to increasing the protein concentration alone indicates the reassignment of nutrients towards growth, rather than maintenance. Further evidence was observed from decreasing the meat lipid contents, the ALT and ALT/AST ratios and the uric acid, compared to feeding the high-protein diet alone. These results indicate enhanced protein utilisation for growth as a result of increasing energy availability for net protein utilisation. Similar results were reported by GHAZALAH et al. (2008) and BRAKE et al. (1998). In addition, increasing the protein and energy concentration significantly decreased thymus percentage compared to increasing the protein concentration alone, while increasing the PA compared to the thermoneutral group showed improved cell-mediated immunity of the broilers.

Conclusion

CHS decreased growth, feed, protein and energy intake and impaired conversion of protein, energy and feed, survival rate and EPI, while increasing the meat lipid percentage. The chronic heat stress group significantly increased AST compared to the other groups. In addition, CHS significantly elevated plasma cholesterol and glucose, cloaca temperature and respiration rate compared to the thermoneutral group. The PCV and bursa percentage was significantly decreased due to CHS. SOD was significantly decreased due to CHS exposure, compared to the thermoneutral groups, but catalase, TAC, IgA, IgM and IgG were significantly increased.

Increasing protein with or without energy concentration improved growth, feed and protein utilisation, survival rate and EPI, PCV, TAC and IgG, hepatic cellular permeability markers (AST and alkaline phosphatase) and stress index (cortisone), hence partially relieving the negative effects on growth and feed utilisation. However, increasing both protein and energy concentration was more effective for production and EPI, meat lipid and blood uric acid than increasing the protein concentration alone.

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Conflict of interest

The authors certify that they have no conflict of interest in the subject matter or materials discussed in this manuscript.

Summary

A total of 140, 28-day-old male Ross-308 broiler chickens were used in a straight-run experimental design. The broilers were distributed among 4 treatment groups. One treatment group was raised under a thermoneutral condition (28 ± 4°C and 40 –60% RH) and fed on a basal diet containing 19% CP and 13.18 MJ ME/kg diet during days 28–49 (thermoneutral group). The other three groups (2, 3 and 4) were raised for 4 successive days per week under 36 ± 3°C and 40–60% RH for 6 h per day from 10 a.m. to 4 p.m. Group 2 was kept under chronic heat stress and fed on the basal diet and was considered a negative control. Group 3 was kept under chronic heat stress and fed feed having 22% CP and 13.18 MJ/ME kg, while group 4 was kept under chronic heat stress and fed on a diet containing 22% CP and 13.81 MJ ME/kg. Exposure to chronic heat stress decreased BWG and increased meat lipids, the liver leakage marker AST, cholesterol, glucose, cortisone and alkaline phosphatase, cloacal temperature, respiration rate, PCV, bursa percent, catalase, TAC and IgA, IgM and IgG compared to the thermoneutral group, but showed decreased SOD. on the other hand, increasing the protein concentration alone or in combination with increasing the energy concentration by increasing oil supplementation from 4.5% to 7.5% relieved the negative effects of chronic heat stress in broiler performance, meat lipids and physiological and immunological traits. Increasing the protein with energy concentration was the most effective method. Thus, it can be concluded that increasing protein with energy concentration may be a useful tool in improving the productive performance, meat quality, blood haematological and biochemical traits, antioxidants and immunity of broiler chickens.

Key words

Broiler, nutrition, heat stress, protein, energy, growth, carcass, blood constituents, immunity.

Zusammenfassung

Hitzetoleranz von Broilern bei Fütterung von Rationen mit variierenden Protein- und Energiegehalten

In der Untersuchung wurden 140 Ross 308 Broiler ab dem 28. Lebenstag verwendet. Die Broiler wurden hierzu auf 4 Behandlungen verteilt. Eine thermoneutral gehaltene Gruppe (1; Haltung bei 28 ± 4°C, 40–60% RF; Basisfutter mit 19% RP und 13.2 MJ ME/kg) diente als Gesamtkontrolle. Die Versuchsgruppen (2, 3, 4) wurden an vier aufeinander folgenden Tagen über 6 Stunden (10:00–16:00 h) einem Hitzestress (36 ± 3°C, 40–60% RF) ausgesetzt. Gruppe 2 wurde mit dem Basisfutter gefüttert (negative Kontrolle), Gruppe 3 erhielt ein Futter mit 22% RP und 13,2 MJ ME/kg und Gruppe 4 erhielt ein Futter mit 22% RP und 13.8 MJ ME/kg. Der Versuch wurde in der Periode 28. bis 49. Lebenstag durchgeführt.

Chronischer Hitzestress verminderte die Zunahmen und erhöhte den Gehalt an Lipiden im Fleisch. Ferner waren die Rektaltemperatur die Respirationsrate, das PCV, das relative Gewicht der Bursa-Drüse sowie die Spiegel des Leberfunktionsindikators AST, von Cholesterol, Glukose, Cortison, Alkaline Phosphatase, Catalase, TAC, IgA, IgM und IgG im Blut im Vergleich zur thermoneutralen Gruppe erhöht, während der SOD-Siegel vermindert war. Demgegenüber wirkte sich eine alleinige Erhöhung des Proteingehaltes oder des Energiegehaltes im Futter (Steigerung des Fettgehaltes von 4,5 auf

7,5%) bei den Hitzestress-Gruppen nicht negativ auf die Leistung, den Lipidgehalt des Fleisches und die immunologischen Parameter aus. Die kombinierte Erhöhung des Protein- und des Energiegehaltes im Futter erwies sich als effektivste Methode. Es wurde daher der Schluss gezogen, dass bei unter Hitzestress gehaltenen Tieren eine gleichzeitige Erhöhung des Protein- und Energiegehaltes im Futter die Mastleistung, die Fleischqualität sowie die hämatologischen, biochemischen, anti-oxidativen und immunologischen Parameter im Blut verbessern kann.

Stichworte

Broiler, Fütterung, Hitzestress, Protein, Energie, Wachstum, Schlachtkörper, Blutbestandteile

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Correspondence: Y.A. Attia, Arid Land Agriculture Department, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, P.O. Box 80208, Jeddah 21589, Saudi Arabia; E-mail: yaattia@kau.edu.sa