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The effects of protein, amino acid, and dietary electrolyte balance on broiler chicken performance and blood parameters under heat stress

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ABSTRACT. The effect of crude protein (CP), amino acid (AA), and dietary electrolyte balance (DEB) were evaluated on blood parameters, carcass traits, and broiler performance under heat stress (29-34°C). A total of 540 male chickens (Ross 308) were allocated to 12 diets with factorial arrangement 2 × 2 × 3, using a completely randomized design with three replicates of 15 chickens in grower (13 to 26 days) and finisher (27 to 42 days) periods. and 120, 220, and 320 mEq kg^{-1} DEB. The level of 21% CP increased body weight gain (BWG) and decreased feed conversion ratio (FCR) at grower period ($p < 0.05$). In contrast, 20% CP level decreased BWG and increased FCR at finisher period ($p < 0.05$). Further, 20% CP level reduced blood sodium and blood electrolyte balance ($p < 0.05$). The highest blood electrolyte balance was achieved by DEB 320 mEq kg^{-1} diet ($p < 0.05$). Broiler response to DEB in heat stress depended on the age of bird, length of exposure to high temperature and CP level of the diet. Under heat stress (29-34°C), the 21% CP level at grower period and 17% CP level at finisher period improved broiler BWG and FCR.

Keywords: standard and additional of digestible amino acid, heat stress, carcass traits.

Efeitos de proteína, amino ácido e balanço alimentar eletrolítico no desempenho de aves de corte e parâmetro sanguíneos sob estresse calórico

RESUMO. O efeito de proteína bruta (PB), amino ácido (AA) e balanço alimentar eletrolítico (DAE) foi avaliado por parâmetros sanguíneos, características da carcaça e desempenho de aves de corte sob estresse calórico (29-34°C). Quinhentos e quarenta machos (Ross 308) foram alocados em 12 regimes alimentares num arranjo fatorial 2 × 2 × 3 totalmente aleatório com 3 repetições de 15 aves em períodos de crescimento (13 a 26 dias) e terminação (27 a 42 dias) e 120, 220 e 320 mEq kg^{-1} DAE. Além disso, 21% de PB aumentou o ganho de peso corporal (GPC) e diminuiu a conversão alimentar (CAR) no período de crescimento ($p < 0.05$). Por outro lado, 20% de PB diminuiu GPC e aumentou CAR no período terminal ($p < 0.05$). O nível de 20% PB reduziu o sódio sanguíneo e o balanço eletrolítico sanguíneo ($p < 0.05$). O maior balanço eletrolítico sanguíneo ocorreu com DAE 320 mEq kg^{-1} dieta ($p < 0.05$). A resposta das aves de corte a DAE no estresse calórico dependia da idade da ave, à exposição a altas temperaturas e ao nível de PB na dieta. Sob estresse calórico (29-34°C), 21% PB no período de crescimento e 17% de PB no período de terminação melhoraram o GPC e CAR das aves de corte.

Palavras-chave: amino ácido padrão e adicional, estresse calórico, características da carcaça.

Introduction

Nutrition and metabolism play an important role in the acid-base balance of broiler chickens (Borges, Silva, Maiorka, Hooge, & Cummings, 2004; Olanrewaju, Wongpichet, Thaxton, Dozier, & Branton, 2006) and eventually performance. Heat stress is one of the most problems in tropical regions to broiler production. Monovalent electrolytes, i.e., sodium (Na^+), potassium (K^+), and chloride (Cl^-) are the key minerals, which consider as dietary electrolyte balance (DEB, $Na^+ + K^+ - Cl^-$) (Mongin, 1981; Borges, Silva, Ariki, Hooge, & Cummings, 2003a), have the greatest impact on acid-base balance or pH of blood and tissues under heat stress (Borges et al., 2004). High temperature accomplish with negative balance of minerals led to broiler respiratory alkalosis, decreased feed intake and growth performance (Belay & Teeter, 1993). The birds under heat stress fed diets with DEB of around 250 mEq kg^{-1} showed better performance, well maintained blood physiological parameters [pH, HCO_3, pCO_2, hemoglobin (Hb), hematocrit (H), lymphocytes (L), and H to L ratio] and blood nutrients (glucose, Na, K and Cl) and also retained more strong ions (Na, K and Cl) (Ahmad & Sarwar, 2006). On the other hand, the growth responses of broilers that consuming diets containing low CP at optimal requirement levels of limited AA (Lys, Met, and Thr) had similar to broiler performance fed diets based on National Research Council (NRC, 1994) recommendations (Safamehr, Narimani, & Nobakht, 2012). Alternatively, DEB can affect nutrient metabolism, especially AA, in broiler (Murakami, Rondon, Martins, Pereira, & Scapinello, 2001). Reduction of nitrogen in poultry wastes and reduce environment pollution is primary consideration of these diets. So, CP levels in diets is depends on limited AA which supplemented in the diets. Therefore, it is necessary to be existent a balance among DEB, AA, and CP levels under heat stress to insure that optimal growth and well-being of broiler were attained. Keeping in view the role of strong ions to alleviate heat stress in broiler chicken and their different AA and CP requirements in this situation, a few publications investigated their interaction on broiler performance. Thus, an experiment was carried out to study the effects of crude protein, supplemental amino acids, and dietary electrolyte balance levels on some blood parameters, carcass traits and broiler performance under heat stress condition.

Material and methods

The experimental methods regarding Animal Care were approved by the Animal Ethics Committee of the Bu-Ali Sina University in Iran.

Husbandry, diets, and experimental design

The male broiler chickens (Ross 308) were fed with similar starter diet from 1 to 12 days of age (metabolisable energy, 2,950 kcal kg^{-1} and CP, 21.45%). Experimental basal diet was formulated according to Ross 308 International (2013 – referenced) recommendation (Table 1). A total of 540 birds were allocated to 12 experimental diets consisted of three factor of CP (2 levels), AA (2 levels), and DEB (3 levels) with arranged a factorial using a completely randomized design (CRD). Each treatment was replicated 3 times with 15 chickens. Two periods: 1- grower (13-26 days of age) and finisher (27-42 days of age) were defined under heat stress condition (29-34°C) in all phases.

Table 1. Composition of experimental diets at grower period (13-27 days of age, %).

Crude protein level	19			19			21			21		
Amino acid[1]	Standard			Additional			Standard			Additional		
DEB[2] (mEg kg^{-1})	120	220	320	120	220	320	120	220	320	120	220	320
Corn grain	53.750	53.750	53.750	53.750	53.750	53.75	49.200	49.200	49.200	49.200	49.200	49.200
Wheat	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Soybean meal (44%)	29.450	29.450	29.450	29.450	29.450	29.450	32.000	32.000	32.000	32.000	32.000	32.000
Fish meal	1.000	1.000	1.000	1.000	1.000	1.000	3.020	3.020	3.020	3.020	3.020	3.020
Soybean Oil	1.500	1.500	1.500	1.500	1.500	1.500	2.080	2.080	2.080	2.080	2.080	2.080
CBC[3]	1.210	1.210	1.210	1.210	1.210	1.210	0.995	0.995	0.995	0.995	0.995	0.995
MCP[4]	1.220	1.220	1.220	1.220	1.220	1.220	1.010	1.010	1.010	1.010	1.010	1.010
Vit and min Premix[5]	0.200	0.200	0.200	0.200	0.200	0.20	0.200	0.200	0.200	0.200	0.200	0.200
Met (99%)	0.245	0.245	0.245	0.285	0.285	0.285	0.202	0.202	0.202	0.241	0.241	0.241
Lys (74.4)	0.190	0.190	0.190	0.255	0.255	0.255	0.033	0.033	0.033	0.100	0.100	0.100
Thr (98.5%)	0.050	0.050	0.05	0.082	0.080	0.080	0	0	0	0.020	0.020	0.020
NaCl	0.160	0.160	0.160	0.160	0.160	0.160	0.130	0.130	0.130	0.130	0.130	0.130
$NaHCO_3$	0.170	0.170	0.170	0.170	0.170	0.170	0.130	0.130	0.130	0.130	0.130	0.130
$KHCO_3$	-	-	0.675	-	-	0.680	-	-	0.500	-	-	0.525
NH_4Cl	0.550	0.015	-	0.550	0.010	-	0.685	0.150	-	0.665	0.128	-
Inert	0.305	0.840	0.180	0.285	0.830	0.155	0.315	0.850	0.500	0.209	0.746	0.249
Analyzed composition												
AME_n (kcal kg^{-1})	2890	2890	2890	2890	2890	2890	2890	2890	2890	2890	2890	2890
CP	19.270	19.270	19.270	19.270	19.270	19.270	20.960	20.960	20.960	20.960	20.960	20.960
Ca	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826
Available P	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413	0.413
Na	0.147	0.147	0.147	0.147	0.147	0.147	0.148	0.148	0.148	0.148	0.148	0.148
K	0.800	0.800	1.18	0.800	0.800	1.182	0.851	0.851	1.123	0.851	0.851	1.147
Cl	0.545	0.190	0.180	0.545	0.187	0.18	0.591	0.236	0.137	0.590	0.235	0.150
Dig[6] Arg	1.013	1.013	1.013	1.098	1.098	1.098	1.245	1.245	1.245	1.2445	1.245	1.245
Dig Lys	1.012	1.012	1.012	1.060	1.060	1.060	1.009	1.009	1.009	1.060	1.060	1.060
Dig Met	0.505	0.505	0.505	0.544	0.544	0.544	0.493	0.493	0.493	0.531	0.531	0.531
Dig Met + Cys	0.770	0.770	0.770	0.809	0.809	0.809	0.771	0.771	0.771	0.809	0.809	0.809
Dig Isol	0.719	0.719	0.719	0.723	0.723	0.723	0.792	0.792	0.792	0.792	0.792	0.792
Dig Thr	0.670	0.67	0.670	0.703	0.703	0.703	0.687	0.678	0.687	0.707	0.707	0.707
Dig Val	0.793	0.793	0.793	0.809	0.809	0.809	0.870	0.870	0.870	0.870	0.870	0.870
Dig Try	0.194	0.194	0.194	0.194	0.194	0.194	0.213	0.213	0.213	0.213	0.213	0.213
Final DEB diet (mEq kg^{-1})	120	220	320	120	220	320	120	220	320	120	120	320

[1]The 19% CP used with AA standard [% - Methionine (Met): 0.245, Threonine (Thr): 0.500, and Lysine (Lys): 0.190] and AA additional (Met: 0.285, Thr: 0.800, and Lys: 0.255). The 21% CP used with AA standard (Met: 0.202, Thr: 0.000, and Lys: 0.033) and AA additional (Met: 0.241, Thr: 0.020, and Lys: 0.100); [2]Dietary Electrolyte Balance; [3]Calcium bi carbonate; [4]Mono calcium phosphate; [5]Vitamin and mineral Premix: mineral supplement (kg) containing: Mn (oxide) 99200, Zn (oxide) 84700, Fe (sulfate) 5000, Copper (sulfate) 10000, Iodine 992, Selenium 200, and Calcium chloride 500000 mg; vitamin supplement (kg) containing: A 900000, D3 2000000, and E 18000 IU; B 11703, B3 6600, B5 29700, B6 2955, B12 15, H2 100, and Antioxidant 100000 mg; [6]Digestible.

For grower period, 19% CP with digestible AA standard including [Methionine (Met): 0.245, Threonine (Thr): 0.500, and Lysine (Lys): 0.190%] and digestible AA additional levels including (Met: 0.280, Thr: 0.800, and Lys: 0.250%) as well as 21% CP with AA standard (Met: 0.200, Thr: 0.000, and Lys: 0.030%) and additional levels (Met: 0.240, Thr: 0.020, and Lys: 0.100%), and dietary electrolyte balance (DEB) 120, 220, 320 mEq kg^{-1} was used (Table 1). Moreover, The 17% CP with digestible AA standard (Met: 0.180, Thr: 0.030, and Lys: 0.150%) and additional levels (Met: 0.210, Thr: 0.060, and Lys: 0.210%) and 20% CP with AA standard (Met: 0.110, Thr: 0.000, and Lys: 0.000%) and digestible additional levels (Met: 0.150, Thr: 0.000, and Lys: 0.000%), and DEB 120, 220, 320 mEq kg^{-1} were used in finisher period (Table 2).

Feed and water were offered *ad libitum*. Light was provided for 24 hour day^{-1} and gradually was reduced to 23 hour day^{-1}, and temperature was gradually reduced 3°C from the initial 32°C in each week. Feed intake (FI) and body weight gain (BWG) were measured weekly. Thus feed conversion ratio (FCR) was calculated.

Carcass traits

On 42 days of age, two birds from each replicate were randomLy selected, tagged, and weighted, then fasted for 8 hours (no limitation of water access) and were slaughtered. Carcass weight was measured after removal of feather, head, legs and abdominal contents. The abdominal fat dissected and recorded. The breast and thigh weights were calculated as the percentage of live body weights.

Blood parameters

On 42 days of age, two chickens selected randomLy from each replicate (n = 3) and blood taken from their wing vein and then immediately transferred on ice to laboratory. Serum samples were taken (centrifuged 3,500 × rpm for 15 min) and blood elements (Na, K, and Cl) were measured by ion selective electrode method of electrolyte analysis system (Kartium, Model E921-XI).

Table 2. Composition of experimental diets at finisher period (27-42 days of age, % or as is).

Crude protein level	17			17			20			20		
Amino acid[1]	Standard			Additional			Standard			Additional		
DEB[2] (mEg kg^{-1})	120	220	320	120	220	320	120	220	320	120	220	320
Corn grain	49.500	49.500	49.500	49.500	49.500	49.500	42.700	42.700	42.700	42.700	42.700	42.700
Wheat	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000	20.000
Soybean meal (44%)	24.630	24.630	24.630	24.630	24.630	24.630	28.720	28.720	28.720	28.720	28.720	28.720
Fish meal	0	0	0	0	0	0	3.000	3.000	3.000	3.000	3.000	3.000
Soybean Oil	1.750	1.750	1.750	1.750	1.750	1.750	2.480	2.480	2.480	2.480	2.480	2.480
CBC[3]	1.255	1.255	1.255	1.255	1.255	1.255	0.940	0.940	0.940	0.940	0.940	0.940
MCP[4]	1.210	1.210	1.210	1.210	1.210	1.210	0.900	0.900	0.900	0.900	0.900	0.900
Vit and min Premix[5]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Met (99%)	0.181	0.181	0.181	0.214	0.214	0.214	0.112	0.112	0.112	0.147	0.147	0.147
Lys (74.4)	0.149	0.149	0.149	0.213	0.213	0.213	0	0	0	0	0	0
Thr (98.5%)	0.030	0.030	0.030	0.060	0.060	0.060	0	0	0	0	0	0
NaCl	0.175	0.175	0.175	0.175	0.175	0.175	0.125	0.125	0.125	0.125	0.125	0.125
$NaHCO_3$	0.180	0.180	0.180	0.180	0.180	0.180	0.120	0.120	0.120	0.120	0.120	0.120
$KHCO_3$	-	0.118	0.675	-	0.145	0.700	-	-	0.440	-	-	0.437
NH_4Cl	0.235	-	-	0.317	-	-	0.519	0.090	-	0.518	0.091	-
Inert	0.406	0.623	0.065	0.269	0.468	-	0.184	0.613	0.263	0.153	0.577	0.231
Analyzed composition												
AME_n kcal kg^{-1}	2925	2925	2925	2925	2925	2925	2925	2925	2925	2925	2925	2925
CP	17.370	17.370	17.370	17.370	17.37	17.370	20.110	20.110	20.110	20.110	20.110	20.110
Ca	0.777	0.777	0.777	0.777	0.777	0.777	0.777	0.777	0.777	0.777	0.777	0.777
NPP	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.384	0.384
Na	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146	0.146
K	0.726	0.793	1.10	0.726	0.808	1.12	0.808	0.808	1.056	0.808	0.808	1.054
Cl	0.404	0.182	0.182	0.146	0.195	0.195	0.475	0.191	0.131	0.475	0.191	0.131
Dig[6] Arg	0.995	0.995	0.995	0.995	0.995	0.995	1.710	1.710	1.710	1.710	1.710	1.710
Dig Lys	0.854	0.854	0.854	0.902	0.902	0.902	0.920	0.920	0.920	0.920	0.920	0.920
Dig Met	0.414	0.414	0.414	0.447	0.447	0.447	0.393	0.393	0.393	0.427	0.427	0.427
Dig Met + Cys	0.667	0.667	0.667	0.701	0.701	0.701	0.667	0.667	0.667	0.667	0.667	0.667
Dig Isol	0.627	0.627	0.627	0.627	0.627	0.627	0.749	0.749	0.749	0.749	0.749	0.749
Dig Thr	0.576	0.576	0.576	0.605	0.605	0.605	0.648	0.648	0.648	0.648	0.648	0.648
Dig Val	0.709	0.709	0.709	0.691	0.691	0.691	0.829	0.829	0.829	0.829	0.829	0.829
Dig Try	0.175	0.175	0.175	0.175	0.175	0.175	0.205	0.205	0.205	0.205	0.205	0.205
Final DEB diet (mEq kg^{-1})	120	220	320	120	220	320	120	220	320	120	220	320

[1]The 17% CP were used with AA standard [% - Methionine (Met): 0.181, Threonine (Thr): 0.030, and Lysine (Lys): 0.149] and AA additional (Met: 0.214, Thr: 0.060, and Lys: 0.213) and the 20% CP used with AA standard (Met: 0.112, Thr: 0.000, and Lys: 0.000) and AA additional (Met: 0.147, Thr: 0.000, and Lys: 0.000); [2]Dietary Electrolyte Balance; [3]Calcium bi carbonate; [4]Mono calcium phosphate; [5]Vitamin and mineral Premix: mineral supplement (kg) containing: Mn (oxide) 99200, Zn (oxide) 84700, Fe (sulfate) 5000, Copper (sulfate) 10000, Iodine 992, Selenium 200, and Calcium chloride 500000 mg; vitamin supplement (kg) containing: A 900000, D3 2000000, and E 18000 IU; B 11703, B3 6600, B5 29700, B6 2955, B12 15, H2 100, and Antioxidant 100000 mg; [6]Digestible.

In addition, one extra chicken was selected from each replicate and 2 (for blood gases assay) and 1 mL (for blood cell count) blood were taken from their wing vein and transferred into heparin tubes. Blood cell counts, hemoglobin and hematocrit (Hk) was measured using cell counter (Model, ERMA-Japan). In addition, Gimsa stain was used to heterophile (H), and lymphocytes (L) measurement. Moreover, blood gas (CO_2, O_2, and HCO_3) concentrations were determined using 9950IVL system (Switzerland).

Statistical analysis

All data were analyzed with CP levels, AA levels, and DEB as factorial 2 × 2 × 3 using a completely randomized design by the GLM procedure of Statistical Analysis System (SAS, 2004). Significant differences were compared by Duncan's multiple range test ($p \leq 0.05$).

Results

The effects of experimental diets on broiler performance at grower (13- 26 days of age) and finisher (27- 42 days of age) periods are presented in Tables 3 and 4, respectively. The 21% CP level increased BWG and improved FCR at grower period ($p < 0.05$). In contrast, BWG decreased and FCR increased by 20% CP at finisher period ($p < 0.05$). Other parameters did not affected by AA and DEB levels ($p < 0.05$) (Table 4). The effects of treatments on carcass traits of broiler chickens at 42 days are presented in Table 5. The results indicated that the level of 20% CP increased carcass percentage ($p < 0.05$) but other parameters or traits were not affected by AA and DEB levels. The results of diets on blood parameters of broiler chickens at 42 days of age are presented in Table 6. The results demonstrated that 20% CP level was significantly increased Hb concentration than 17% CP level ($p < 0.05$). Hematological profile of blood is good indicator of stress in birds. The results of blood gas concentration of broiler chickens in response to diets at 42 days of age are given in Table 7. The results indicated that CP, AA, and DEB levels had no significantly effects on blood pH, P_{O2}, P_{CO2}, and HCO_3 concentrations. Increased respiration rates induced by severe heat stress seemed necessary for evaporative cooling in broilers. The results of blood ingredient concentration of broiler chickens at 42 days of age are presented in Table 8. The results have shown that 20% CP level significantly reduced Na concentration and electrolyte balance than 17% CP level ($p < 0.05$). The highest electrolyte balance was achieved by the level of DEB 320 mEq kg^{-1} other than treatments ($p < 0.05$).

Table 3. The effect of treatments on broiler performance at grower (13-26 days of age).

	BWG[1] (g day^{-1})	FI[2] (g)	FCR[3]	Mortality (%)
CP[4] Level (%)				
19	67.46 [b]±4.23	1139.38±9.49	2.07 [a]±0.15	2.47±5.69
21	71.74 [a]±5.74	1138.27±6.19	1.93 [b]±0.13	4.12±6.84
p value	0.03	0.70	0.01	0.48
SEM	1.32	2.07	0.03	1.65
AA[5] level				
Standard[6]	69.17±5.99	137.85±8.36	2.00±0.18	2.47±5.69
Additional[7]	70.02±4.93	139.80±7.56	2.00±0.13	4.12±6.84
p value	0.65	0.51	0.99	0.48
SEM	1.32	2.07	0.03	1.65
DEB[8] Level				
120	68.59±5.90	139.39±7.78	2.04±0.16	3.71±6.72
220	71.57±4.31	137.70±7.55	1.92±0.14	2.47±5.78
320	68.64±5.81	139.79±8.83	2.04±0.14	3.71±6.72
p value	0.34	0.76	0.10	0.88
SEM	1.62	2.54	0.04	2.02
p value				
CP×AA	0.69	0.46	0.80	0.99
CP×DEB	0.79	0.76	0.76	0.42
AA×DEB	0.81	0.64	0.58	0.88
CP×AA×DEB	0.99	0.65	0.81	0.69

[1]Body weight gain; [2]Feed intake; [3]Feed conversion ratio; [4]Crude protein; [5]Amino acid; [6]The AA standard according to Ross 308 International recommendation (Aviagen, 2013) [Methionine (Met): 0.240, Threonine (Thr): 0.500, and Lysine (Lys): 0.190] for 19% CP and AA standard (Met: 0.200, Thr: 0.000, and Lys: 0.030) for 21% CP; [7]AA additional (Met: 0.280, Thr: 0.800, and Lys: 0.250) for 19% CP and AA additional (Met: 0.240, Thr: 0.020, and Lys: 0.100) for 21% CP; [8]Dietary Electrolyte Balance.

Table 4. The effect of treatments on broiler performance (27-42 days of age).

	BWG (g)	FI (g)	FCR	Mortality (%)
CP[4] Level (%)				
17	100.27 [a]±8.70	193.43±8.16	1.93 [b]±0.13	2.26±5.44
20	93.69 [b]±9.56	196.53±10.77	2.12 [a]±0.27	4.73±6.90
p value	0.03	0.35	0.02	0.26
SEM	2.13	2.31	0.05	1.48
AA[5] level				
Standard[6]	96.76±9.02	193.18±7.08	2.01±0.20	3.13±6.05
Additional[7]	97.20±10.42	196.78±11.42	2.04±0.26	3.96±6.58
p value	0.88	0.28	0.66	0.69
SEM	2.13	2.31	0.05	1.48
DEB[8] Level				
120	94.59±9.32	192.03±9.08	2.04±0.17	3.55±6.43
220	100.68±9.07	196.74±6.29	1.97±0.21	3.63±6.57
320	95.68±10.07	196.14±12.40	2.07±0.30	3.46±6.29
p value	0.23	0.45	0.53	0.99
SEM	2.61	2.83	0.06	1.81
p value				
CP*AA	0.83	0.75	0.99	0.69
CP*DEB	0.48	0.82	0.43	0.22
AA*DEB	0.48	0.68	0.38	0.11
CP*AA*DEB	0.12	0.14	0.56	0.57

[1]Body weight gain; [2]Feed intake; [3]Feed conversion ratio; [4]Crude protein; [5]Amino acid; [6]The AA standard according to Ross 308 International recommendation (Aviagen, 2013), (Met: 0.180, Thr: 0.030, and Lys: 0.150) for 17% CP and AA standard (Met: 0.110, Thr: 0.000, and lys: 0.000) for 20% CP; [7]AA additional (Met: 0.210, Thr: 0.060, and Lys: 0.210) for 17% CP and AA additional (Met: 0.150, Thr: 0.000, and Lys: 0.000) for 20% CP; [8]Dietary Electrolyte Balance.

Table 5. The effects of dietary treatments on carcass traits (% of × live body weight) and abdominal fat (% of body weight) at 42 days of age.

	Carcass	Thighs	Breast	Abdominal fat
CP[1] Level (%)				
17	62.27^{b}±1.25	34.38±2.16	38.00±3.50	1.59±0.46
20	63.22^{a}±1.58	24.70±2.08	37.14±3.66	1.48±0.38
p value	0.03	0.53	0.34	0.08
SEM	0.22	64.19	0.55	0.06
AA[2] level				
Standard[3]	62.44±1.36	34.65±1.99	37.57±3.91	1.51±0.44
Additional[4]	63.05±1.58	34.43±25.2	37.57±3.28	1.50±0.40
p value	0.10	0.53	0.66	0.93
SEM	0.22	64.19	0.55	0.06
DEB[5] Level (mEq kg^{-1})				
120	63.00±1.41	34.81±2.15	36.80±3.15	1.66±0.45
220	62.66±1.60	34.10±1.84	38.08±3.40	1.59±0.42
320	62.58±1.50	34.70±2.33	37.82±4.15	1.36±1.35
p value	0.80	0.67	0.65	0.37
SEM	0.27	78.63	0.68	0.08
p value				
CP*AA	0.99	0.67	0.65	0.37
CP*DEB	0.54	0.67	0.41	0.38
AA*DEB	0.83	0.67	0.51	0.78
CP*AA*DEB	0.32	0.68	0.68	0.16

[a, b]Means with different superscripts in each part of each same column are different ($p < 0.05$), µ±SD: Means±standard deviation, SEM = Standard error of the means, [1]Crude Protein; [2]Amino Acid; [3]According to Ross 308 International recommendation (Aviagen, 2013), AA standard (Met: 0.181, Thr: 0.030, and Lys: 0.149) for 17% CP and AA standard (Met: 0.112, Thr: 0.000, and Lys: 0.000) for 20% CP; [4]AA additional (Met: 0.214, Thr: 0.060, and Lys: 0.213) for 17% CP and AA additional (Met: 0.147, Thr: 0.000, and Lys: 0.000) for 20% CP; [5]Diet Electrolyte Balance.

Table 6. The effect of treatments on blood parameters of broilers at 42 days of age.

	Hemoglobin (g dl^{-1})	Hematocrit (%)	Heterophile – Lymphocytes ratio
CP[1] Level (%)			
17	16.88^{b}±0.79	32.25±3.61	0.06±0.05
20	17.87^{a}±1.42	34.23±3.10	0.06±0.03
p value	0.02	0.10	0.77
SEM	0.29	0.82	0.007
AA[2] level			
Standard[3]	17.25±3.69	33.17±3.69	0.07±0.05
Additional[4]	17.51±1.38	33.31±3.33	0.05±0.03
p value	0.52	0.91	0.08
SEM	0.29	0.82	0.007
DEB[5] Level			
120	17.40±1.04	32.67^{ab}±3.54	0.072^{ab}±0.05
220	16/87±1.26	31.78^{b}±2.99	0.078^{a}±0.04
320	17.86±1.31	35.27^{a}±3.10	0.044^{b}±0.02
p value	0.16	0.05	0.05
SEM	0.35	1.01	0.009
p value			
CP*AA	0.39	0.85	0.40
CP*DEB	0.96	0.96	0.66
AA*DEB	0.87	0.65	0.09
CP*AA*DEB	0.18	0.97	0.001

[a, b]Means with different superscripts in each part of each same column are different ($p < 0.05$), µ±SD: Means±standard deviation, SEM = Standard error of the means, [1]Crude Protein; [2]Amino Acid; [3]According to Ross 308 International recommendation (Aviagen, 2013), AA standard (Met: 0.181, Thr: 0.030, and Lys: 0.149) for 17% CP and AA standard (Met: 0.112, Thr: 0.000, and Lys: 0.000) for 20% CP; [4]AA additional (Met: 0.214, Thr: 0.060, and Lys: 0.213) for 17% CP and AA additional (Met: 0.147, Thr: 0.000, and Lys: 0.000) for 20% CP; [5]Diet Electrolyte Balance.

Table 7. Blood gas concentrations (mm hg) of broiler chickens in response to diets at 42 days of age.

	pH	P_{CO2}	P_{O2}	HCO_3
CP[1] Level (%)				
17	7.42±0.08	46.21±10.96	66.57±29.86	28.46±3.53
20	7.40±0.07	46.42±10.63	62.15±17.52	28.12±5.23
p value	0.51	0.95	0.61	0.82
SEM	0.01	0.14	6.11	1.05
AA[2] level				
Standard[3]	7.40±0.06	46.93±9.89	68.57±24.28	28.91±5.33
Additional[4]	7.41±0.09	45.70±11.60	60.15±24.12	27.67±3.25
p value	0.76	0.73	0.33	0.41
SEM	0.01	0.14	6.11	1.05
DEB[5] Level (mEq kg^{-1})				
120	7.41±0.07	44.00±10.46	56.76±14.75	26.35±4.01
220	7.38±0.09	51.13±12.33	74.85±26.33	29.55±5.04
320	7.44±0.06	43.81±7.81	61.46±11.79	28.96±3.71
p value	0.23	0.18	0.22	0.19
SEM	0.02	3.10	7.48	1.28
p value				
CP*AA	0.76	0.90	0.85	0.83
CP*DEB	0.31	0.29	0.40	0.74
AA*DEB	0.72	0.27	0.99	0.14
CP*AA*DEB	0.45	0.56	0.87	0.59

µ±SD: Means±standard deviation, SEM = Standard error of the means, [1]Crude Protein; [2]Amino Acid; [3]According to Ross 308 International recommendation (Aviagen, 2013), AA standard (Met: 0.181, Thr: 0.030, and Lys: 0.149) for 17% CP and AA standard (Met: 0.112, Thr: 0.000, and Lys: 0.000) for 20% CP; [4]AA additional (Met: 0.214, Thr: 0.060, and Lys: 0.213) for 17% CP and AA additional (Met: 0.147, Thr: 0.000, and Lys: 0.000) for 20% CP; [5]Diet Electrolyte Balance.

Table 8. The effect of diets on blood constituents of broiler chickens at 42 days of age (mmoL L^{-1} or mentioned).

	Sodium	Potassium	Chloride	Blood electrolyte balance (mEq kg^{-1})
CP[1] Level (%)				
17	152.08^{a}±6.90	4.45±0.64	113.01±5.33	43.52^{a}±3.52
20	148.28^{b}±4.32	4.69±0.69	111.30±5.21	41.64^{b}±2.96
p value	0.02	0.18	0.29	0.02
SEM	1.15	0.003	1.11	0.54
AA[2] level				
Standard[3]	149.92±6.60	4.57±0.71	112.57±5.70	41.92±3.54
Additional[4]	150.41±5.49	4.57±0.64	111.74±4.91	43.24±3.10
p value	0.76	0.97	0.60	0.10
SEM	1.15	0.003	1.11	0.54
DEB[5] Level (mEq kg^{-1})				
120	148.15±4.34	4.66±0.64	111.25±3.75	41.57^{b}±2.75
220	151.10±4.23	4.35±0.63	113.66±5.47	41.80^{b}±3.06
320	151.24±8.32	4.70±0.71	111.56±6.25	44.37^{a}±3.61
p value	0.23	0.23	0.41	0.01
SEM	1.41	0.15	1.37	1.42
p value				
CP*AA	0.36	0.39	0.21	0.41
CP*DEB	0.23	0.82	0.25	0.25
AA*DEB	0.39	0.56	0.30	0.17
CP*AA*DEB	0.75	0.76	0.42	0.58

[a, b]Means with different superscripts in each part of each same column are different ($p < 0.05$), µ±SD: Means±standard deviation, SEM = Standard error of the means, [1]Crude Protein; [2]Amino Acid; [3]According to Ross 308 International recommendation (Aviagen, 2013), AA standard (Met: 0.181, Thr: 0.030, and Lys: 0.149) for 17% CP and AA standard (Met: 0.112, Thr: 0.000, and Lys: 0.000) for 20% CP; [4]AA additional (Met: 0.214, Thr: 0.060, and Lys: 0.213) for 17% CP and AA additional (Met: 0.147, Thr: 0.000, and Lys: 0.000) for 20% CP; [5]Diet Electrolyte Balanc.

Discussion

By using modern broiler chickens, the suitable DEB for starter and grower period reported 246-315 and 249-257 mEq kg^{-1}, respectively (Murakami et al., 2001) which are in contrast with results of current study. The results are in agreement with the findings of Temim et al. (1999), who reported that broilers feeding with high protein diets (25 vs. 20%) had higher BWG during grower period under heat stress condition (32°C). Because of the different levels of protein and strain of broiler, changes in nutrition requirements and metabolism, similar results did not observe in finisher period. Balnave and Oliva (1991) reported that maximum BWG and FI observed at 30°C with DEB 380 mEq kg^{-1}. Under moderate environmental condition, However, the effects of different DEB (87, 180, 280 and 380 mEq kg^{-1}) treatments were non-significant. Borges, Silva, Maiorka, Hooge, and Cummings (2004) reported that DEB of 240 was most favorable for broilers in either temperature environment (22.5±3.5°C; and cyclic stress 22.5±3.5°C for 14 hours and 33±2.0°C for 10 hours). Borges, Silva, Ariki, Hooge, & Cummings (2003a) reported that FI linearly increase with increasing of DEB (40-340 mEq kg^{-1}). Conflicting findings emphasized that the effects of DEB on broiler performance is diverse and seems depends on differences in basal diet, broiler strain, temperature of rearing and supplemented electrolyte salts. Moreover, increasing of CP levels with essential AA in 32°C temperature increased FI in male broiler chickens (Alleman & Leclercq, 1997) that is in contrast with present study. This inconsistency could be related to differences in amount of CP levels and severity of heat stress condition. On the other hand, Flemming et al. (2001) compared three different levels of high, modulate, and low DEB in broiler chickens during the summer, and indicated that DEB had no effect on BWG. In addition, Borges et al. (2003a) pointed out that diets with DEB 240 mEq kg^{-1} was showed the best BWG than diets with DEB 0, 120, and 360 mEq kg^{-1} in broiler chicken at 23-30ºC. In this study, using 21% CP level in grower period and 17% CP level in finisher period had significant improvement on FCR than 19 and 20% CP levels in grower and finisher periods, respectively ($p < 0.05$). The results of present study are match with findings of Flemmnig et al. (2001) that FCR did not affect by various DEB levels. However, Borges et al. (2003a) showed that 240 mEq kg^{-1} DEB had best FCR comes before DEB than 120 and 360 mEq kg^{-1} in broiler chickens under 30-32°C. The results showed that different CP, AA, DEB levels, and their interaction had not significantly effect on mortality. Results are in agreement of other researchers (Flemming et al., 2001; Borges et al., 2003a), which no significant mortality was found by different DEB in broiler chickens under various temperature condition.

Kamran and Mirza (2004) observed that broiler carcass percentage significantly increased by fed diet with 20% CP in compared to 21, 22, and 23% CP levels. These results are in contrast with present study. No significant differences were obtained in other traits. Ahmad and Sarwar (2005) showed that different sources of DEB supplemented with sodium bicarbonate increased significantly carcass weights, which supported the finding of current study. It is reported that DEB 40-340 mEq kg^{-1} had no effects on abdominal fat and carcass percentage (Borges et al., 2003a) which are inconsistence with the results of present study. Different experimental period (grower period) and severity of heat stress could explain to some extent of this inconsistency. Moreover, the similar breast and thighs percentage were reported in broiler chickens fed diets supplemented with Lys at 15-49 days of age (Holsheimer & Ruesink, 1993).

The differential leukocyte count and the monitoring of its changes in relation to effects of heat stress are an important part of the hematological profile of broilers (Ahmad & Sarwar, 2005). In contrast with the results of present study, the finding of Safamehr, Narimani, and Nobakht (2012) indicated that different levels of CP had no significant effect on broiler blood Hb concentration. Furthermore, 320 mEq kg^{-1} level DEB increased Hk percentage than 220 mEq kg^{-1} level but decreased H to L ratio ($p < 0.05$). No significant differences were observed on other parameters. Water loss by evaporation through respiration in heat stress induced an increase in plasma volume (hemodilution), that caused blood Hk and Hb values to be lowered down. Any therapy that increases blood Hk and Hb values in heat stress will be beneficial (Ahmad & Sarwar 2005). Therefore, increasing DEB up to 320 mEq kg^{-1} level could help to bearing of heat stress to broilers. The H and H to L ratio have been indicated to be reliable indicator to measure of stress (Zulkifli & Siegel, 1995). Under heat stress the H increase and L decrease. Maxwell and Robertson (1998) pointed out that moderate exposures to most stressors caused an increase in the number of H, and the H to L ratio in chickens. In contrast, Borges, Silva, Ariki, Hooge, and Cummings (2003b) reported significantly low H to L ratio for DEB 240 than those of 40, 140 and 340 DEB in both thermoneutral and heat stressed rooms. The results of current study are in contrast with the finding of Safamehr et al. (2012). These could be due to differences in DEB levels, intensity of heat stress, or bird. Increasing blood Hk was matched with results of other researchers (Zhou, Fujita, & Yamamoto, 1999; Vecerek, Strakova, Suchy, & Voslarova, 2002; Bedanova, Voslarova, Vecerek, Strakova, & Suchy, 2003). This increase in Hk concentration is due to DEB increases.

During severe panting, decreased blood partial pressure of CO_2 and, blood HCO_3 concentration but increased pH resulted in respiratory alkalosis and blood acid - base perturbations (Teeter, Smith, & Owens, 1985; Raup & Bottje, 1990). Present results indicated that during panting phase, blood pH was not as much as worse by treatments and it remained relatively constant in the range of 7.38 to 7.44. In contrast to the present findings, Teeter, Smith, and Owens (1985) reported that blood pH was elevated by chronic heat stress (32°C), and broilers had intermittent respiratory alkalosis during panting.

It is reported that broiler chickens fed diets with DEB 360 mEq kg^{-1} increased blood pH, and HCO_3 rather DEB 0, 120, 240 mEq kg^{-1} without any significant effects on blood P_{CO2} and P_{O2} under relatively heat stress and relative moisture condition at 42 days of age (Borges et al,. 2003a). The results of blood CO_2 is in agreement but the results of blood pH, $P_{O2,}$ and HCO_3^- are in contrast with the findings Borges et al. (2003a). Moreover, DEB 360 mEq kg^{-1} increased pH and blood HCO_3 rather 0, 120 and 240 mEq kg^{-1} without any affection on blood P_{CO2} and this inconsistency could be due to variation in the type of broiler chickens and severity of heat stress. Consequently, at high temperature, birds increase their respiration rate in an attempt to increase the rate of evaporative cooling. Such hyperventilation increases CO_2 losses and as a result respiratory alkalosis develops (Belay & Teeter, 1993) increase pH blood, and thereby increase blood CO_2 as well as decrease blood HCO_3 (Borges et al., 2003a).

Khone and Jones (1975) reported that an increase in blood K level in response to heat stress. This K response seems to be related to the time which birds are under stress. It is reported DEB affected Cl^- levels under heat stress as serum Cl^- increased with increasing DEB levels (Ahmad and Sarwar, 2005). These observations are opposite of the results of present study which could be because of different DEB and age of broiler chickens. No significant difference were observed regarding Cl, Na, and K levels of blood serum with increasing DEB from 40 to 340 mEq kg^{-1} (Borges et al., 2003a). These results confirmed by the results of present study at finisher period. Moreover, in opposite of current study the different CP levels significantly changed Na on blood elements (Safamehr et al., 2009). In addition, DEB at levels 147 to 247 mEq kg^{-1} and calcium carbonate supplementation increased in blood electrolyte balance concentration (Olanrewaju et al., 2006) that is in according to results of present study.

Conclusion

Under heat stress challenges, broiler chickens used compensatory mechanisms when attempting to maintain acid-base homeostasis and concentrations of blood nutrients and physiological variables. These reactions make difficult to explain more precisely interaction of CP, AA, and DEB in the heat stress condition. However, an identical DEB 320 mEq kg^{-1} with 21% CP at grower and 17% CP at finisher period gave better carcasses traits and performance parameters with more reduction of sever of heat stress (H to L ratio). The present study indicates that AA levels had no obvious interaction with DEB and CP levels.

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References

Ahmad, T., & Sarwar, M. (2005). Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. *Animal Feed Science and Technology, 120*(3-4), 277-298.

Ahmad, T., & Sarwar, M. (2006). Dietary electrolyte balance: implications in heat stressed broilers. *World's Poultry Science Journal, 62*(4), 638-653.

Alleman, F., & Leclercq, B. (1997). Effects of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *Journal of British Poultry Science, 38*(5), 607-610.

Aviagen Technical Service Manager. (2013). *Ross broiler management manual*. Alabama, AL: Aviagen.

Balnave, D., & Oliva, A. G. (1991). The influence of sodium bicarbonate and sulfur amino acids on the performance of broilers at moderate and high temperatures. *Australian Journal of Agricultural Research, 42*(8), 1385-1397.

Bedanova, I., Voslarova, E., Vecerek, V., Strakova, E., & Suchy, P. (2003). The haematological profile of broilers under acute and chronic heat stress at 30±1°C level. *Folia Veterinaria, 47*(4), 188-192.

Belay, T., & Teeter, R. G. (1993). Broiler water balance and thermohalance during Thermoneutral and high ambient temperature exposure. *Poultry Science Journal, 72*(1), 116-124.

Borges, S. A., Silva, A. V. F., Ariki, J., Hooge, D. M., & Cummings, K. R. (2003a). Dietary electrolyte balance for broiler chickens under moderately high ambient temperatures and relative humidities. *Poultry Science Journal, 82*(2), 301-308.

Borges, S. A., Silva, A. V. F., Ariki, J., Hooge, D. M., & Cummings, K. R. (2003b). Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. *Poultry Science Journal, 82*(3), 428-435.

Borges, S. A., Silva, A. V. F., Maiorka, A., Hooge, D. M., & Cummings, K. R. (2004). Effects of diet and cyclic daily heat stress on electrolyte, nitrogen and water intake, excretion and retention by colostomized male broiler chicken. *International Journal of Poultry Science, 3*(5), 313-321.

Flemming, J. S., Arruda, J. S., Souza, G. A., Fedalta, L. M., France, S. G., Fleming, R., ... Peron, I. (2001). Influence of dietary electrolyte balance on the performance traits of broiler. *Archive Veterinary Science, 6*(3), 89-96.

Holsheimer, J. P., & Ruesink, E. W. (1993). Effect of performance, carcass composition, yield and financial return of dietary energy and lysine levels in starter and finisher diet fed to broiler. *Poultry Science Journal, 72*(5), 806-815.

Kamran, Z., & Mirza, M. (2004). Effect of decreasing dietary protein levels with optimal aminoacids profile on the performance of broilers. *Pakistan Veterinary Journal, 24*(4), 165-168.

Khone, H. J., & Jones, J. G. (1975). Changes in plasma electrolytes acid-base balance and other physiological parameters of adult female turkeys under conditions of acute hyperthermia. *Poultry Science Journal, 54*(6), 2034-2038.

Maxwell, M. H., & Robertson, G. W. (1998). The avian heterophil leukocyte: a review. *World Poultry Science Journal, 54*(2), 155-178.

Mongin, P. (1981). Recent advances in dietary cation-anion balance: Applications in poultry. *Proceeding of Nutrition Society, 40*(3), 285-294.

Murakami, A. E., Rondon, E. O. O., Martins, E. N., Pereira, M. S., & Scapinello, C. (2001). Sodium and chloride requirements of growing broiler chickens (twenty-one to forty-two days of age) fed corn soybean diets. *Poultry Science Journal, 80*(3), 289-294.

National Research Council. (1994). *Nutrient requirements of poultry* (9th ed.). Washington, DC: National Academy Press.

Olanrewaju, H. A., Wongpichet, S., Thaxton, J. P., Dozier, W. A., & Branton, S. L. (2006). Stress and acid-base balance in chickens. *Poultry Science Journal, 85*(7), 1266-1274.

Raup, T. J., & Bottje, W. G. (1990). Effect of carbonated water on arterial pH, pCO_2 and plasma lactate in heat-stressed broilers. *Brittish Poultry Science, 31*(2), 377-384

Safamehr, A., Narimani, M., & Nobakht, A. (2012). Effects of electrolyte balance and dietary protein levels on production performance and carcass parameters in broiler chickens exposed to heat stress. *Journal of Veterinary Research,* 67(3), 297-306.

Statistical Analysis System. (2004). *SAS® user's guide: statistics*. Cary, NC: SAS Institute Inc.

Teeter, R. G., Smith, M. O., & Owens, F. N. (1985). Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. *Poultry Science Journal, 64*(6), 1060-1064.

Temim, S., Chagneau, A. M., Guillaumin, S., Michael, J., Peresson, R., Geraert, P. A., & Tesseraud, S. (1999). Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. *Representative Nutrition Development, 39*(1), 145-156.

Vecerek, V., Strakova, E., Suchy, P., & Voslarova, E. (2002). Influence high environmental temperature on production and haematological and biochemical indexes in broiler chickens. *Czech Journal of Animal Science,* 47(5), 176-182.

Zhou, W. T., Fujita, M., & Yamamoto, S. (1999). Thermoregulatory responses and blood viscosity in dehydrated heat exposed broilers (Gallus Domesticus). *Journal of Thermal Biology, 24*(3), 185-192.

Zulkifli, I., & Siegel, P. B. (1995). Is there a positive side to stress? *World's Poultry Science Journal, 51*(1), 63-67.

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