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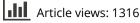
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# Effects of different dietary electrolyte balances on growth performance, carcass traits, blood parameters and immune responses of broilers

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#### ABSTRACT

The effects of different dietary electrolyte balances (DEB; 220, 230, 240, 250, 260, 270 and 280 mEq/kg of diet) were investigated on growth performance, carcass traits, blood serum parameters and immune responses of broilers under thermoneutral environment. Data were analysed in agreement to a completely randomized design. In the grower period (linear, P = .01) and throughout the study (linear, P < .01) feed, energy and protein intakes decreased by increasing the DEB level. In the starter period, ADG showed the lowest value at 250 DEB level (quadratic, P < .01), whereas in the grower period and throughout the study, ADG decreased (linear, P < .01) by increasing the DEB level. Serum uric acid showed the lowest concentration at 250 DEB level (quadratic, P = .02). The serum triglyceride concentration decreased (linear, P = .02) and concentration of high-density lipoproteins raised (linear, P < .01) by increasing DEB level. Immune response against Gumboro disease tended (P = .09) to raise (linear, P < .01) by increasing the DEB level. No considerable DEB effect was observed on carcass traits and relative organ weights. The DEB values ranging from 220 to 240 mEq/kg could be appropriate to promote broiler growth performance.

ARTICLE HISTORY

Received 18 February 2019 Accepted 17 November 2021

#### **KEYWORDS**

Acido-base homeostasis; anatomical parts; antibody response; carcass yield; feed efficiency; poultry

# Introduction

In birds, acid-base balance is affected by some factors, such as environmental condition, diet and metabolism, which alter the regulation of pH in the blood and tissues (Olanrewaju et al., 2007; Adekunmisi and Robbins, 2009). The monovalent ions Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> play a crucial role in maintaining chicken's acid-base homeostasis: Na<sup>+</sup> and K<sup>+</sup> are alkalogenic, whereas Cl<sup>-</sup> is acidogenic.

The value of dietary electrolyte balance (DEB) is commonly calculated by the difference between the sum of monovalent cations (Na<sup>+</sup> + K<sup>+</sup>) and monovalent anion (Cl<sup>-</sup>), and expressed as mEq/kg of diet (Mongin, 1981). The DEB value is one of the main factors that influence the broiler performance (Shafey et al., 2011). Diets with a low DEB cause metabolic acidosis and vice versa, diets with a high DEB bring to alkalosis. Metabolic acidosis or alkalosis may adversely affect feed intake, body weight gain and feed efficiency, and therefore animal productivity (Ahmad et al., 2009).

In poultry nutrition, DEB of 250 mEq/kg is generally recommended for normal physiological function (Mongin, 1981) and salts of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> (such as sodium carbonate, potassium chloride, sodium chloride, ammonium chloride and potassium sulphate) are commonly added to diets. More recently, conflicting DEB levels have been reported as adequate to guarantee optimal broiler performance. Borges et al. (2003a, 2003b) reported a DEB range of 220–240 mEq/kg as adequate and especially, a DEB value of 240 mEq/kg as the best, under high ambient temperatures. Szabó et al. (2011) reported that, in controlled environmental conditions, diets with 175 mEq/kg DEB promote good broiler performance until 21 days of age, but DEB should be 250 mEq/kg during the grower and the finisher phases.

The literature data call attention to the question that the ideal DEB level for maximum broiler performance could depend on broiler age and environmental conditions; therefore, further research must be conducted in this sense.

The aim of this study was to investigate the effects of different DEB levels on growth performance, carcass traits, blood serum parameters and the immune of broilers in a thermoneutral environment.

#### Materials and methods

#### Birds and housing

All procedures were approved by the Animal Care and Welfare Committee of Islamic Azad University.

A total of 280 1-day-old Ross 308 male broilers (Aviagen, Newbridge, Scotland, UK) were weighed (initial BW of  $44.0 \pm 1.3$  g) and randomly allocated to seven dietary treatments. Each treatment was assigned to four replicate floor cages with 10 birds per cage. The birds were reared in floor cages (10 birds/m<sup>2</sup>) in an environmentally controlled room until the end of the experiment (42 days). Room temperature was maintained at 32°C for the first week of age thereafter was maintained at 24°C by a tunnel ventilation. Relative humidity was maintained at 55–65% by a water

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This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. spray. The lighting programme throughout the study was of 23 h lighting and 1 h darkening. The feed was provided on the plastic tray during the first 2 weeks of the trial then through appropriate feeders. Each cage was equipped with automatic drinkers. Water and experimental diets were offered *ad libitum*.

# different types of salts (NaHCO<sub>3</sub>, KHCO<sub>3</sub> and NH<sub>4</sub>Cl). All experimental diets were formulated to be iso-energetic and isonitrogenous, and to meet or exceed the nutritional requirements of broilers as suggested by Ross (2007) for starter and grower periods. Ingredients, chemical composition and energy of broiler diets for starter and grower periods are shown in Tables 1 and 2.

# **Dietary treatments**

Broiler responses to different DEB were evaluated from 1 to 21 days (starter period), from 22 to 42 days (grower period) and throughout the study (from 1 to 42 days). The treatments consisted of different levels of DEB (220, 230, 240, 250, 260, 270 and 280 mEq/kg of diet). The levels were obtained by adding

#### Growth performance

Bodyweight and feed intake (difference of offered feed and refused feed) were measured weekly. The average daily feed intake (ADFI), average daily gain (ADG), gain to feed ratio (G: F), average daily energy intake (ADEI), gain to metabolizable

Table 1. Ingredients, chemical composition and energy of the broiler diets for starter period (from 1 to 21 days of age).

ltem				DEB (mEq/kg of diet	:)		
Ingredients (g/kg)	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB
Corn	552.10	553.60	554.50	556.00	556.70	555.00	553.30
Soybean meal	371.80	371.50	371.30	371.00	371.00	371.20	371.60
Soybean oil	29.80	29.30	29.00	28.50	28.30	28.90	29.40
Dicalcium phosphate	19.40	19.40	19.40	19.40	19.40	19.40	19.40
DL-Methionine	01.30	1.30	1.30	1.30	1.30	1.30	1.30
L-Lysine	0.40	0.40	0.40	0.40	0.40	0.40	0.40
CaĆO <sub>3</sub>	13.50	13.50	13.50	13.50	13.50	13.50	13.50
NaCl	2.00	2.00	2.00	2.00	2.00	2.00	2.00
NaHCO <sub>3</sub>	2.50	2.40	2.40	2.40	2.40	3.20	4.10
KHCO <sub>3</sub>	_	_	0.10	_	_	0.10	_
NH <sub>4</sub> CI	2.20	1.60	1.10	0.50	_	_	_
Vitamin premix <sup>a</sup>	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Mineral premix <sup>b</sup>	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Calculated chemical composition		2.50	2.50	2.50	2.50	2.50	2.50
Dry matter	864.60	865.10	865.40	865.90	866.40	866.40	866.70
Crude protein	230.00	230.00	230.00	230.00	230.00	230.00	230.00
Crude fat	54.50	54.10	5.38	5.33	5.32	5.37	5.41
Linoleic acid	28.80	28.60	28.50	28.30	28.20	28.40	28.70
Crude fibre	26.70	26.70	26.70	26.70	26.70	26.70	26.70
	10.50					10.50	10.50
Calcium		10.50	10.50	10.50	10.50		
Phosphorus Austicklaukaankaans	7.40	7.40	7.40	7.40	7.40	7.40	7.40
Available phosphorus	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium	9.20	9.20	9.20	9.20	9.20	9.20	9.20
Chloride	3.20	2.80	2.40	2.00	1.70	1.70	1.70
Manganese (mg/kg)	403.61	403.61	403.61	403.61	403.61	403.61	403.61
Sodium	1.70	1.70	1.70	1.70	1.70	1.90	2.10
Zinc (mg/kg)	324.20	324.20	324.20	324.20	324.20	324.20	324.20
Choline (mg/kg)	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Folic acid	21.10	21.10	21.10	21.10	21.10	21.10	21.10
Arginine	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Glycine	9.40	9.40	9.40	9.40	9.40	9.40	9.40
Serine	11.30	11.30	11.30	11.30	11.30	11.30	11.30
Glycine+Serine	24.40	24.40	24.40	24.40	24.40	24.40	24.40
Histidine	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Isoleucine	9.50	9.50	9.50	9.50	9.50	9.50	9.50
Leucine	19.40	19.40	19.40	19.40	19.40	19.40	19.40
Lysine	12.70	12.70	12.70	12.70	12.70	12.70	12.70
Methionine	4.80	4.80	4.80	4.80	4.80	4.80	4.80
Cystine	3.70	3.70	3.70	3.70	3.70	3.70	3.70
Methione+Cystine	8.40	8.40	8.40	8.40	8.40	8.40	8.40
Phenylalanine	10.80	10.80	10.80	10.80	10.80	10.80	10.80
Tyrosine	8.90	8.90	8.90	8.90	8.90	8.90	8.90
Phenylalanine +Tyrosine	19.70	19.70	19.70	19.70	19.70	19.70	19.70
Threonine	8.60	8.60	8.60	8.60	8.60	8.60	8.60
Tryptophan	3.10	3.10	3.10	3.10	3.10	3.10	3.10
Valine	10.50	10.50	10.50	10.50	10.50	10.50	10.50
ME <sup>c</sup> (kcal/kg)	3025	3025	3025	3025	3025	3025	3025
DEP diatary alactrolyta balanc		5025	5025	5025	5025	5025	5625

DEB, dietary electrolyte balance.

<sup>a</sup>Supplied the following per kilogram of diet: retinyl acetate, 2.7 mg; cholecalciferol, 42.5 mg; dl-alpha-tocopheryl acetate, 10 mg; menadione sodium bisulphite, 2 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; niacin, 20 mg; cyanocobalamin, 0.01 mg; folacin, 0.5 mg; pyridoxine, 1.5 mg; biotin, 0.1 mg; choline chloride, 200 mg; ethoxyguin, 125 mg: bacitracin, 10 mg.

<sup>b</sup>Supplied the following per kilogram of diet: Mn, 80 mg; Zn, 75 mg; Fe, 20 mg; Cu, 5 mg; I, 1.2 mg; Co, 0.2 mg; Se, 0.3 mg; NaCl, 2,500 mg; NaSO<sub>4</sub>, 1000 mg. <sup>c</sup>Metabolizable energy of the diets estimated using the Carpenter and Clegg's equation (Leeson and Summers, 2001)

Table 2. Ingredients, chemical composition and energy of the broiler diets for grower period (from 22 to 42 day of age).
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ltem	DEB (mEq/kg of diet)									
Ingredients (g/kg)	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB			
Corn	595.60	596.60	59.75	59.56	59.39	59.21	59.05			
Soybean meal	323.80	323.60	32.34	32.38	32.41	32.44	32.47			
Soybean oil	41.00	40.70	4.04	4.10	4.16	4.22	4.27			
Dicalcium phosphate	17.00	17.00	1.70	1.70	1.70	1.70	1.70			
DL-Methionine	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
L-Lysine	_	-	-	_	-	_	-			
CaĆO <sub>3</sub>	11.30	11.30	11.30	11.30	11.30	11.30	11.30			
NaCl	2.00	2.00	2.00	2.00	2.00	2.00	2.00			
NaHCO <sub>3</sub>	2.30	2.30	2.30	2.70	3.90	4.40	5.60			
KHCO <sub>3</sub>	_	_	0.10	0.60	0.20	0.60	0.20			
NH₄CI	1.00	0.50	_	_	-	_	_			
Vitamin premix <sup>a</sup>	2.50	2.50	2.50	2.50	2.50	2.50	2.50			
Mineral premix <sup>b</sup>	2.50	2.50	2.50	2.50	2.50	2.50	2.50			
Calculated chemical composit		2.50	2.50	2.50	2.50	2.50	2.50			
Dry matter	870.80	871.20	871.50	871.20	871.80	871.50	872.10			
Crude protein	210.00	210.00	210.00	210.00	210.00	210.00	210.00			
Crude fat	66.90	66.60	66.30	66.90	67.40	67.90	68.40			
Linoleic acid	35.30	35.20	35.00	35.30	35.60	35.90	36.10			
Crude fibre	25.70	25.70	25.70	25.70	25.70	25.70	25.70			
	9.00		9.00		9.00	9.00				
Calcium		9.00		9.00			9.00			
Phosphorus Austilation and a sub-	6.80	6.80	6.80	6.80	6.80	6.80	6.80			
Available phosphorus	4.50	4.50	4.50	4.50	4.50	4.50	4.50			
Potassium	8.40	8.40	8.40	8.60	8.40	8.60	8.40			
Chloride	2.30	1.90	1.60	1.60	1.60	1.60	1.60			
Manganese (mg/kg)	400.39	400.39	400.39	400.39	400.39	400.39	400.39			
Sodium	1.60	1.60	1.60	1.60	1.60	1.60	1.60			
Zinc (mg/kg)	322.23	322.23	322.23	322.23	322.23	322.23	322.23			
Choline (mg/kg)	1.47	1.47	1.47	1.47	1.47	1.47	1.47			
Folic acid	19.50	19.50	19.50	19.50	19.50	19.50	19.50			
Arginine	13.50	13.50	13.50	13.50	13.50	13.50	13.50			
Glycine	8.60	8.60	8.60	8.60	8.60	8.60	8.60			
Serine	10.20	10.20	10.20	10.20	10.20	10.20	10.20			
Glycine+Serine	22.10	22.10	22.10	22.10	22.10	22.10	22.10			
Histidine	5.50	5.50	5.50	5.50	5.50	5.50	5.50			
Isoleucine	8.60	8.60	8.60	8.60	8.60	8.60	8.60			
Leucine	18.10	18.10	18.10	18.10	18.10	18.10	18.10			
Lysine	11.10	11.10	11.10	11.10	11.10	11.10	11.10			
Methionine	4.20	4.20	4.20	4.20	4.20	4.20	4.20			
Cystine	3.40	3.40	3.40	3.40	3.40	3.40	3.40			
Methione+Cystine	7.60	7.60	7.60	7.60	7.60	7.60	7.60			
Phenylalanine	9.80	9.80	9.80	9.80	9.80	9.80	9.80			
Tyrosine	8.10	8.10	8.10	8.10	8.10	8.10	8.10			
Phenylalanine +Tyrosine	17.90	17.90	17.90	17.90	17.90	17.90	17.90			
Threonine	7.80	7.80	7.80	7.80	7.80	7.80	7.80			
Tryptophan	2.80	2.80	2.80	2.80	2.80	2.80	2.80			
Valine	9.60	9.60	9.60	9.60	9.60	9.60	9.60			
ME <sup>c</sup> (kcal/kg)	3150	3150	3150	3150	3150	3150	3150			

DEB, dietary electrolyte balance.

<sup>a</sup>Supplied the following per kilogram of diet: retinyl acetate, 2.7 mg; cholecalciferol, 42.5 mg; dl-alpha-tocopheryl acetate, 10 mg; menadione sodium bisulphite, 2 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; niacin, 20 mg; cyanocobalamin, 0.01 mg; folacin, 0.5 mg; pyridoxine, 1.5 mg; biotin, 0.1 mg; choline chloride, 200 mg; ethoxyquin, 125 mg; bacitracin, 10 mg.

<sup>b</sup>Supplied the following per kilogram of diet: Mn, 80 mg; Zn, 75 mg; Fe, 20 mg; Cu, 5 mg; I, 1.2 mg; Co, 0.2 mg; Se, 0.3 mg; NaCl, 2,500 mg; NaSO<sub>4</sub>, 1000 mg. <sup>c</sup>Metabolizable energy of the diets estimated using the Carpenter and Clegg's equation (Leeson and Summers 2001).

energy ratio (G:ME), average daily protein intake (ADPI) and gain to protein intake ratio (G:P) were calculated for each replicate within the treatment and for the periods d 0 to d 21 (starter period), d 22 to d 42 (grower period) and d 0 to d 42 (whole period).

#### **Blood serum parameters**

At the end of the study (d 42), blood samples were collected from four birds per treatment (1 bird/replicate group) by wing vein. Blood samples (1 ml/bird) were collected into EDTA tubes, centrifuged (3000 rpm  $\times$  10 min) at room temperature. Then, plasma was stored at  $-20^{\circ}$ C until analysis. Biochemical analyses [uric acid, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and alkaline phosphatase (ALP)] were performed using standard protocols of commercial laboratory kits (Pars Azmoon Co., Tehran, Iran) as described by Golrokh et al. (2016).

#### Immune response

Birds were vaccinated against: avian influenza virus (AI) and infectious bronchitis (strain H120) at 1 day of age (by eyedrop application); Gumboro disease at 19 days of age (oral route by drinking water) and Newcastle disease (NDV, strain *Viscerotropic velogenic*) at 1 (by oral-spray), 5, 8 and 21 days (oral route by drinking water) of age.

Table 3. Growth performance of Ross 308 broilers fed diets with different DEB (mEq/kg of diet) levels in starter (d0 to d21) and grower (d22 to d42) periods, and throughout the study (d0 to d42).

				Treatment			Contrasts				
	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB	SEM	P-value	Linear	Quadratic
ADFI (g)											
d0 to d21	48.13	48.48	48.18	46.30	46.77	47.33	47.57	0.692	.29	0.15	0.20
d22 to d42	165.20	159.37	164.88	165.64	158.97	158.86	158.75	1.641	.01	0.01	0.35
d0 to d42	106.67	103.93	106.53	105.97	102.87	103.09	103.16	0.873	.01	<0.01	0.72
ADG (g)											
d0 to d21	34.65	34.21	34.98	32.15	33.65	34.05	34.43	0.372	<.01	0.25	<0.01
d22 to d42	80.30	79.68	79.89	79.63	79.49	76.35	77.23	0.865	.03	<0.01	0.27
d0 to d42	57.48	56.94	57.44	55.89	56.57	55.20	55.83	0.467	.02	<0.01	0.82
G.F											
d0 to d21	0.72	0.71	0.73	0.69	0.72	0.72	0.72	0.010	.24	0.55	0.23
d22 to d42	0.49	0.50	0.48	0.48	0.50	0.48	0.49	0.005	.20	0.37	0.74
d0 to d42	0.60	0.60	0.61	0.59	0.61	0.60	0.61	0.006	.21	0.88	0.37
ADEI (kcal)											
d0 to d21	145.60	146.66	145.75	140.06	141.47	143.17	143.90	2.094	.29	0.15	0.20
d22 to d42	520.39	502.02	519.37	521.77	500.75	500.40	500.06	5.171	.01	0.01	0.35
d0 to d42	332.99	324.34	332.56	330.92	321.11	321.78	321.98	2.73	.01	<0.01	0.70
G:ME (g/kcal)											
d0 to d21	0.24	0.23	0.24	0.23	0.24	0.24	0.24	0.003	.24	0.55	0.23
d22 to d42	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.001	.20	0.37	0.74
d0 to d42	0.20	0.20	0.20	0.19	0.20	0.20	0.20	0.002	.21	0.88	0.37
ADPI (g)											
d0 to d21	11.07	11.15	11.08	10.64	10.76	10.89	10.94	0.159	.29	0.15	0.20
d22 to d42	34.69	33.47	34.62	34.78	33.38	33.36	33.34	0.345	.01	0.01	0.35
d0 to d42	22.88	22.31	22.85	22.72	22.07	22.12	22.14	0.186	.01	<0.01	0.76
G:P											
d0 to d21	3.13	3.07	3.16	3.02	3.13	3.13	3.15	0.041	.24	0.55	0.23
d22 to d42	2.31	2.38	2.30	2.29	2.38	2.29	2.32	0.021	.20	0.37	0.74
d0 to d42	2.72	2.72	2.73	2.65	2.75	2.71	2.73	0.025	.20	0.92	0.39

DEB, dietary electrolyte balance; ADFI, average daily feed intake; ADG, average daily gain; ADEI, average daily energy intake; G:ME, gain to metabolizable energy intake ratio; ADPI, average daily protein intake; G:P, gain to protein intake ratio.

Significance was considered at  $P \le .05$  and tendency was declared at  $.05 < P \le .10$ .

Briefly, four birds per dietary treatment (1 bird/replicate group) were randomly chosen and blood samples were collected from the brachial vein. Blood serum was separated by centrifugation ( $3000 \text{ rpm} \times 10 \text{ min}$ ) and antibody titres against Gumboro disease and infectious bronchitis were measured, at 38 and 24 days, respectively, after the injection, by using commercially available ELISA kits (Bio-check BV, Gouda, Holland) according to the manufacturer's instructions. The sample absorbance was read at 405 nm using an ELISA reader (Bio-Tek Instruments Inc., ELX 800; Winooski, VT, USA). Within 28 days after the injection, antibody titres against Al and NDV were also measured by hemagglutination-inhibition test according to Cunningham (1971).

#### **Carcass measurements**

At the end of the study (d 42), four birds per dietary treatment (one bird/replicate group) were carefully chosen on the basis of average body weight of the group, killed by cervical dislocation and properly processed to evaluate carcass traits. The carcasses were weighed before and after the removal of head and drumsticks. Viscera and abdominal fat were then removed and the carcass yields and the relative weights (expressed as a percentage of eviscerated carcass) of abdominal fat, anatomical parts (breast, drumsticks, wings, head, neck and notarium), entrails (ventriculus, heart, kidneys and pancreas) and gut tracts (duodenum, jejunum, ileum, colon, right caecum and left caecum) were calculated. The relative weights of organs related to the immune system (thymus, liver, spleen and bursa of Fabricius) were also calculated in accordance with Shabani et al. (2015).

# Statistical analysis

Data were tested for normality with the Shapiro–Wilk test before statistical analysis. Data were analysed either in starter and grower periods (i.e. growth performance) or throughout the study (i.e. growth performance, blood serum parameters, immune responses and carcass traits) according to a completely randomized design using the GLM procedure of SAS (2003). The model was:

$$Y_{ij} = \mu + \text{Treatement}_i + e_{ij},$$

where  $Y_{ij}$  is the response variable,  $\mu$  the overall mean, Treatement<sub>i</sub> the fixed effect of DEB levels (*i* = 7) and  $e_{ij}$  the random residual error.

The IML procedure of SAS (2003) was used to generate contrast coefficients to evaluate linear or quadratic response of DEB levels. Significance was considered at  $P \le .05$  and tendency was declared at  $.05 < P \le .10$ .

#### Results

#### Growth performance

The growth performance of broilers fed diets with different DEB levels is shown in Table 3. In the starter period, there was no treatment effect on ADFI, ADEI and ADPI, whereas in the grower period (linear, P = .01) and throughout the study (linear, P < .01) ADFI, ADEI and ADPI decreased by increasing the DEB level. In the starter period, ADG showed the lowest value at 250 DEB level (quadratic, P < .01), whereas in the grower period and throughout the study (linear, P < .01), ADG

Table 4. Blood serum parameters of Ross 308 broilers (42 days of age) fed diets with different DEB (mEq/kg of diet) levels.

						Contrasts					
	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB	SEM	P-value	Linear	Quadratic
Uric acid (mg/dl)	3.84	4.99	3.93	2.42	5.17	4.88	6.40	0.637	.01	0.02	0.02
Total cholesterol (mg/dl)	214.28	165.46	138.34	169.87	212.06	164.62	201.59	30.969	.54	0.84	0.23
Triglycerides (mg/dl)	116.04	63.01	29.98	65.34	60.70	52.57	33.99	17.614	.05	0.02	0.19
HDL cholesterol (mg/dl)	81.66	83.42	89.95	78.39	95.98	111.88	106.25	4.119	<.01	<0.01	0.09
LDL cholesterol (mg/dl)	105.34	68.49	43.72	73.47	107.98	110.56	79.03	28.625	.61	0.65	0.52
LDL/HDL	1.31	0.85	0.48	0.95	1.12	0.98	0.76	0.297	.58	0.37	0.95
ALP (U/I)	347.70	818.74	386.17	648.03	320.99	384.18	291.32	115.770	.13	0.09	0.17

DEB, dietary electrolyte balance; HDL, high-density lipoproteins; LDL, low-density lipoproteins; ALP, alkaline phosphatase.

Significance was considered at  $P \le .05$  and tendency was declared at  $.05 < P \le .10$ .

decreased by increasing the DEB level. No treatment effects were observed on G:F, G:ME and G:P in the starter and grower periods, and throughout the study.

#### Blood serum parameters and immune response

The effects of different DEB levels on blood serum parameters and immune responses of broilers are shown in Tables 4 and 5. A quadratic treatment effect (P = .02) was found on serum uric acid which showed the lowest concentration at 250 DEB level. Serum triglyceride concentration decreased (linear, P = .02) and HDL concentration raised (linear, P < .01) by increasing DEB level (Table 4).

As shown in Table 5, the immune response against Gumboro disease tended (P = .09) to raise (linear, P < .01) by increasing the DEB level.

#### Carcass traits and relative organ weights

The effects of different DEB levels on carcass traits and relative weights of organs are shown in Table 6. There is no treatment effect on carcass characteristics and relative weights of anatomical parts (except for neck), abdominal fat, entrails, gut tracts and organs related to the immune system. The DEB level affected (tendency, P = .06) the relative weight of the neck which the reduced (linear, P = .01) by increasing the DEB level.

#### Discussion

The results obtained by the current study showed that the DEB level can affect broiler growth performance, blood serum parameters and immune responses without having important effects on carcass traits and relative organ weights. Regarding the growth performance, the DEB level affected intakes (i.e. ADFI, ADEI and ADPI) and weight gain in a different way depending on the growth period. In the starter period (from 1 to 21 days), the DEB level did not affect growth performance, except for the 250 DEB level that reduced broiler weight gain.

In the grower period (from 22 to 42 days) and throughout the study (from 1 to 42 days), broilers showed the highest intakes at the DEB levels of 220, 240 and 250 mEq/kg. Moreover, DEB levels in the range of 220–260 mEq/kg promoted broiler weight gain, except for the 250 DEB level throughout the study as a result of the lowest ADG observed at this DEB level in the starter period.

Amhad et al. (2009), in their study conducted under heat stress conditions, found the enhanced performance of broilers with DEB values of 50, 150 and 250 mEq/kg and concluded that a single DEB value could not be recommended to combat heat stress in broilers. Our results showed that under thermoneutral conditions, DEB values (i.e. from 220 to 240 mEq/kg) lower than that commonly recommended for broilers (250 mEq/kg; Mongin, 1981) seem to be appropriate to promote growth performance in accordance with Borges et al. (2003a,b). In particular, a DEB value of 220 mEq/kg might be considered adequate for both starter and grower periods.

The blood serum results showed that the DEB level can affect the serum concentration of uric acid and the serum lipid profile. In broilers, uric acid is produced as the main end product of N metabolism which in turn depends on the cation–anion balance of the body and therefore on the DEB level (Li et al., 2013). The lowest serum concentration of uric acid was observed at a DEB level of 250 mEq/kg, suggesting that this DEB level could be the most appropriate for N utilization. The serum concentration of triglycerides decreased by passing from acidogenic to alkalogenic DEB levels, whereas

Table 5. Effects of different levels of DEB (mEq/kg of diet) levels on antibody titres against AI, infectious bronchitis, Newcastle disease (NDV) and Gumboro disease.

	Treatment									Contrasts	
	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB	SEM	P-value	Linear	Quadratic
Al <sup>a</sup>	3.00	3.75	3.50	3.50	4.00	3.75	3.00	0.572	.84	0.87	0.23
Bronchitis <sup>b</sup>	3.50	3.50	3.25	3.00	4.50	4.00	3.00	0.485	.31	0.77	0.54
NDV <sup>c</sup>	6.50	6.50	6.00	5.75	5.50	5.50	6.25	0.529	.67	0.26	0.21
Gumboro <sup>d</sup>	3.75	4.00	3.75	4.75	4.75	5.50	5.00	0.463	.09	<0.01	0.86

DEB, dietary electrolyte balance.

<sup>a</sup>Immune response measured within 28 days after injection.

<sup>b</sup>lmmune response measured within 38 days after injection.

<sup>c</sup>Immune response measured within 28 days after injection.

<sup>d</sup>Immune response measured within 24 days after injection.

Significance was considered at  $P \le .05$  and tendency was declared at  $.05 < P \le .10$ .

Table 6. Carcass traits and relative weights (as a percentage of eviscerated carcass) of anatomical parts, abdominal fat, entrails, gut tracts and organs related to
immune system of Ross 308 broilers (42 days of age) fed diets with different dietary electrolyte balance (DEB, mEq/kg of diet) levels.

			Treatment							Contrasts	
	220 DEB	230 DEB	240 DEB	250 DEB	260 DEB	270 DEB	280 DEB	SEM	P-value	Linear	Quadratio
Carcass traits											
Pre-slaughtered BW (g)	2785	2990	2567	2903	2613	2910	2727	212.5	0.76	0.80	0.84
Defeathered carcass weight (g)	2389	2676	2313	2575	2336	2422	2424	218.7	0.90	0.75	0.93
Full carcass weight <sup>a</sup> (g)	2019	2375	2153	2317	2020	2033	2010	231.4	0.84	0.50	0.45
Eviscerated carcass weight (g)	1747	2136	1819	1973	1782	1800	1829	208.8	0.85	0.68	0.67
Carcass yield (%)	72.15	79.73	78.69	76.28	75.75	73.13	75.11	2.527	0.36	0.59	0.18
Anatomical parts											
Breast (%)	27.93	27.19	28.75	26.43	28.20	27.75	26.76	1.258	0.85	0.66	0.79
Drumsticks (%)	25.32	24.34	23.82	17.99	25.97	24.79	24.26	2.305	0.29	0.99	0.22
Wings (%)	6.41	6.77	5.88	6.06	5.34	6.36	6.48	0.377	0.21	0.57	0.07
Neck (%)	2.39	2.22	2.01	1.95	1.86	1.76	2.00	0.138	0.06	0.01	0.06
Head (%)	2.62	2.55	2.58	2.48	2.57	2.32	2.47	0.212	0.96	0.43	0.96
Notarium (%)	4.37	4.55	3.98	3.89	3.75	3.68	4.08	0.300	0.38	0.09	0.22
Abdominal fat and entrails											
Abdominal fat (%)	1.97	1.41	1.65	1.70	2.01	1.89	1.18	0.303	0.44	0.53	0.47
Ventriculus (%)	1.75	1.43	1.43	1.31	1.40	1.30	1.51	0.164	0.53	0.26	0.10
Heart (%)	0.48	0.41	0.42	0.49	0.51	0.51	0.50	0.075	0.93	0.39	0.88
Kidneys (%)	0.69	0.72	0.74	0.53	0.72	0.61	0.68	0.062	0.25	0.45	0.60
Pancreas (%)	0.21	0.18	0.19	0.18	0.20	0.21	0.19	0.018	0.74	0.85	0.49
Gut											
Duodenum (%)	0.73	0.68	0.69	0.63	0.70	0.71	0.73	0.076	0.97	0.86	0.39
Jejunum (%)	1.35	1.23	1.16	1.19	1.19	1.17	0.89	0.154	0.58	0.09	0.67
lleum (%)	1.96	1.60	1.68	1.92	2.37	1.97	1.25	0.462	0.74	0.78	0.38
Colon (%)	0.27	0.21	0.27	0.29	0.35	0.34	0.20	0.071	0.67	0.70	0.33
Right caecum (%)	0.23	0.28	0.23	0.25	0.24	0.25	0.24	0.021	0.65	0.80	0.79
Left caecum (%)	0.22	0.21	0.32	0.21	0.33	0.27	0.23	0.053	0.49	0.63	0.27
Organs related to immune system	n										
Thymus (%)	0.17	0.50	0.54	0.48	0.48	0.15	0.46	0.252	0.85	0.94	0.45
Liver (%)	2.26	1.93	2.29	2.23	2.35	2.17	1.97	0.182	0.59	0.72	0.32
Spleen (%)	0.09	0.10	0.08	0.11	0.08	0.08	0.07	0.014	0.59	0.34	0.41
Bursa of Fabricius (%)	0.05	0.07	0.10	0.10	0.09	0.07	0.03	0.025	0.39	0.56	0.02

DEB, dietary electrolyte balance.

<sup>a</sup>Full carcass deprived of head and drumsticks.

Significance was considered at  $P \le .05$  and tendency was declared at  $.05 < P \le .10$ .

the healthy cholesterol (i.e. HDL cholesterol) increased. However, the best serum lipid profile was observed at 240 DEB level where the lowest triglyceride concentration, as well as the lowest (only numerically) detrimental cholesterol content (i.e. LDL cholesterol) and LDL to HDL ratio were observed.

There is still a lack of information in the literature about the effects of different DEB values on broiler immune responses against AI, infectious bronchitis, NDV and Gumboro disease. Satin et al. (2003) reported that antibody titres against NDV raised linearly by increasing the DEB level (from 40 to 340 mEq/kg). In our study, a similar DEB effect was observed on the immune response against Gumboro disease measured within 24 days after the vaccination. In particular, the immune response against Gumboro disease tended to be higher in broilers fed diets with high-DEB levels (from 250 to 280 mEq/kg) compared with broilers fed diets with low-DEB levels (from 220 to 240 mEq/kg). This result was in agreement with Szabó et al. (2011) that found a positive correlation between the DEB level and the serum antibody titres against Gumboro disease 3 weeks after the immunization. Nevertheless, the same authors observed that this correlation was negative if measured 2 weeks after immunization suggesting that the immune response of broilers fed low-DEB diets (<175 mmol/kg) is faster and more intensive than that of broilers fed medium- or high-DEB diets (>175 mmol/kg). However, the serum antibody levels may persist longer in broilers fed high- than low-DEB diets.

As mentioned above, no considerable DEB effect was observed on broiler carcass traits and relative organ weights (except for neck) in accordance with previous studies (Johnson & Karunajeewa, 1985; Borges et al., 2003a). Nevertheless, Borgatti et al. (2004), in their study on the performance of broilers fed diets with varying DEB levels (210, 250, 290 and 330 mEq/kg) under summer conditions, found DEB effects on the yields of some anatomical parts such as wing, leg and thigh.

# Conclusion

Our findings showed that DEB values (i.e. from 220 to 240 mEq/kg) lower than that commonly recommended (250 mEq/kg) could be appropriate, in both starter and grower periods, to promote broiler growth performance without affecting negatively carcass traits and relative organ weights under thermoneutral environment. Moreover, a DEB level of 240 mEq/kg might improve the lipid profile of blood serum, and a DEB range of 250–280 mEq/kg might increase the broiler immune response against Gumboro disease.

# **Disclosure statement**

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