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Arantes, UM; Stringhini, JH; Oliveira, MC; Martins, PC; Rezende, PM; Andrade, MA; Leandro, NSM; Café, MB

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■Author(s)

Arantes UM^I
Stringhini JH^{II}
Oliveira MC^{III}
Martins PC^{IV}
Rezende PM^V
Andrade MA^{VI}
Leandro NSM^{II}
Café MB^{II}

- Mestre em Ciência Animal, Escola de Veterinária e Zootecnia, UFG, Bolsista do CNPg
- Departamento de Produção Animal, Escola de Veterinária e Zootecnia, UFG, Bolsista do CNPg
- Universidade de Rio Verde, Rio Verde, GO
- Mestranda do curso de Zootecnia do IF Goiano, Rio Verde, GO
- ^v Médico Veterinário UFG, Bolsista do CNPq
- VI Departamento de Medicina Veterinária, Escola de Veterinária e Zootecnia, UFG

■Mail Adress

Corresponding author e-mail address Universidade de Rio Verde, Rio Verde, GO, 75.901-190.

E-mail: mcorv@yahoo.com.br

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ABSTRACT

The aim of this study was to evaluate the performance, tibial density and mineral content, Na, K, and CI serum levels, and dry matter content of the litter of broilers fed diets with different levels of dietary electrolyte balances. Two experiments were carried out: during the starter phase (7) to 21 days of age, 960 broilers) and during the growe phase (22 to 38 days of age, 816 broilers). In both experiments, a completely randomized design with four treatments based on dietary electrolyte balance values (200, 240, 280 and 320 mEq/kg of diet) with four replicates was applied. Birds and diets were weighed when birds were seven, 14 and 21 days of age in the first experiment, and 22 and 38 days of age in the second experiment in order to determine weight gain, feed intake and feed conversion. Final body weight, weight gain, feed intake and feed conversion and mortality rate were evaluated. On days 21 and 38, the left tibia of two birds per replicate was collected to determine bone density and the serum was used for Na and K analysis. Litter dry matter content was also determined on days 21 and 38. There was no effect of dietary electrolyte balance values on broilers performance between 7 and 14, 7 and 21, or 22 and 38 days of age, tibial bone density and mineral content, or on Na, K and Cl serum levels. Litter dry matter was linearly reduced as dietary electrolyte balance value increased. Diets with 200 mEg/kg may be recommended for broilers from 7 to 38 days of age with no negative influence on the evaluated parameters.

INTRODUCTION

High temperatures in the summer pose a considerable challenge to poultry industry, because they negatively affect feed intake, weight gain, carcass weight, and mortality (Teeter & Belay, 1996). Heat stress caused by high temperature increases respiratory rates, resulting in excessive CO₂ loss and respiratory alkalosis, with consequent increase of blood pH (Borges *et al.*, 2003b). In order to correct pH, birds excrete bicarbonate (HCO₃-) through the kidneys. Bicarbonate is a negatively-charged ion that needs to bind to a positively-charged ion, such as Na+ or K+, to be excreted in urine and these ions can be deficient when there is heat stress (Mushtaq *et al.*, 2005).

Dietary electrolyte balance (DEB) is determined by the monovalent chemical elements (Na⁺, K⁺ and Cl⁻), which are considered strong ions because of their effect on the acid-base balance of body fluids (Hooge, 2003). DEB has a crucial role in broiler performance, and it is required for proper bone development and litter quality (Oliveira *et al.*, 2010).

Mongin & Sauveur (1977) determined that the optimal DEB value required for good broiler performance until 28 days of age is 250 mEq/kg, and already proposed the hypothesis that the metabolic



acidosis caused by low DEB diets could affect bone mineralization as a result of alterations in vitamin D metabolism.

Other researchers, however, found DEB different values when evaluating performance, litter quality and bone development. Borges et al. (2003a) reported that the best weight gain and feed conversion of broilers maintained under high temperature and moisture levels was obtained with starter and grower diets with a DEB level 240 mEq/kg. Maiorka et al. (2004) observed during the starter phase, feed intake when dietary DEB was 174 mEq/kg and the best weight gain was obtained with 163 mEq/kg, both statistically better compared with 250 mEq/kg of DEB. According to Szabó et al. (2011), 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.

Vieites *et al.* (2005) evaluated diets with 0 to 350 mEq/kg of DEB and obtained the lowest litter moisture content at 138 and 147 mEq/kg, which corresponded to 26.17% and 2.57%, respectively. Oliveira *et al.* (2010) observed that diets with 200 mEq/kg of DEB for starter and grower broilers promote the best litter moisture and bone development.

Incorrect DEB changes some metabolic pathways, and the electrolytes supplied in feed are used to maintain the homeostasis instead of growth (Mongin, 1981).

Based on this knowledge, this experiment was carried out to evaluate the live performance, tibial density and mineral content, Na, K and Cl serum levels, and litter dry matter content of broilers fed diets with different DEB levels.

MATERIAL AND METHODS

Two experiments were carried out: one in the starter phase (7 to 21 days of age) and the other in the grower phase (22 to 38 days of age). In the first experiment, 960 seven-d-old male Cobb 500 chicks, with 200.83 \pm 2.50g average initial body weight, were allotted in groups of 60 birds per experimental unit, in a total of 16 experimental units. In the second experiment, 816 22-d-old male Cobb 500 broilers, with 941.36 \pm 36.34g of average initial body weight, were allotted in groups of 51 birds per experimental unit, in a total of 16 experimental units.

In both experiments, a completely randomized design with four treatments based on DEB values (200,

240, 280 e 320 mEq/kg of diet) of four replicates each was applied.

During the first seven days of live, chicks were reared according to the recommendations of the genetic line manual (Cobb 500 Manual, 2010). Daily management consisted in cleaning the drinkers and feeders, filling the feeders, and observing any possible mortality. Feed and water were offered *ad libitum*.

The experimental diets were formulated according to the recommendations of Rostagno *et al.* (2005) and differed only relative to DEB specifications. DEB was calculated as the electrolytic ratio (Na++K+-Cl-) of diets, obtained by adding varying levels of Na and Cl in order to achieve the proposed DEB levels of 200, 240, 280 and 320mEg/kg (Table 1).

Birds and diets were weighed when birds were seven, 14 and 21 days of age in the first experiment and 22 to 38 days of age in the second one. These data were used to calculate weight gain, feed intake and feed conversion ratio. Final body weight, weight gain, feed intake, and feed conversion ratio and mortality rate were analyzed.

At 21 and 38 days of age, two birds per replicate were euthanized to obtain the left tibia, which was cleaned for adherent tissues and weighed to determine bone density. Bone density was determined in a 100-mL measuring cylinder using 80 mL of absolute alcohol. Tibia density was measured as:

Tibia density = Tibia weight / (alcohol volume with tibia - alcohol volume without tibia)

On the same days, 3-mL blood samples were collected by heart puncture of two birds per replicate with a sterilized syringe. Samples were identified and centrifuged for five minutes at 1500 rpm for serum separation, which was immediately frozen. Na and K analysis were performed in a flame spectrophotometer using a colorimetric method with commercial reagent kits.

Rice hulls measuring 5cm in height were used as litter material. Litter dry matter content was determined when birds were 21 and 38 days of age using three 500g samples randomly collected from each replicate. Dry matter analysis was performed according to the methodology proposed by Silva & Queiroz (2002).

The obtained results were submitted to analysis of variance (ANOVA) and polynomial regression, using the PROC GLM of the Statistical Analysis System (SAS, 2001).



Table 1 – Ingredients and calculated nutritional composition of the experimental diets fed during the starter and grower phases

Ingredients	Starter phase DEB mEg/kg					Grower phase DEB mEq/kg			
	200	240	280	320	200	240	280	320	
Corn	52.46	53.48	53.00	52.45	54.67	54.27	53.45	52.26	
Soybean meal	32.30	33.50	33.00	32.30	36.00	36.50	37.50	38.60	
Full fat soybeans, 36% CP	8.30	6.50	7.30	8.30	5.80	5.60	4.80	4.10	
Poultry fat	3.00	3.00	3.00	3.00	-	-	-	-	
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.00	1.00	1.00	1.00	
Limestone 37% Ca	1.10	1.10	1.10	1.10	1.20	1.20	1.20	1.20	
Choline chloride 60%	0.54	0.09	0.09	0.09	0.05	0.05	0.05	0.05	
NaCl	0.41	0.35	0.18	0.15	0.42	0.21	0.16	0.15	
DL-methionine 99	0.29	0.29	0.29	0.29	0.31	0.31	0.31	0.31	
KCI	0.00	0.00	0.00	0.23	0.00	0.00	0.54	1.31	
NaHCO ₃	0.00	0.10	0.44	0.50	0.00	0.31	0.44	0.47	
L-lysine HCL	0.10	0.10	0.10	0.10	0.21	0.21	0.21	0.21	
L-threonine	-	-	-	-	0.04	0.04	0.04	0.04	
Mineral/vitamin premix	0.301	0.30^{1}	0.30^{1}	0.30^{1}	0.30^{2}	0.30^{2}	0.30^{2}	0.30^{2}	
Total	100	100	100	100	100	100	100	100	
Calculated composition									
Metabolizable energy (kcal/kg)	3,130	3,130	3,130	3,130	3,298	3,298	3,298	3,298	
Crude protein (%)	22.50	22.50	22.50	22.50	20.80	20.80	20.80	20.80	
Calcium (%)	0.919	0.919	0.919	0.919	0.918	0.918	0.918	0.918	
Available phosphorus (%)	0.475	0.475	0.475	0.475	0.457	0.457	0.457	0.457	
Sodium (%)	0.189	0.191	0.217	0.220	0.194	0.195	0.210	0.210	
Chloride (%)	0.399	0.294	0.193	0.175	0.357	0.231	0.201	0.189	
Potassium (%)	0.936	0.936	0.936	1.066	0.857	0.860	0.957	1.101	
Digestible lysine (%)	1.180	1.180	1.180	1.180	1.108	1.112	1.110	1.110	
Digestible methionine+cystine (%)	0.892	0.889	0.893	0.892	0.581	0.581	0.581	0.581	
Digestible methionine (%)	0.595	0.592	0.596	0.595	0.709	0.711	0.710	0.711	
Digestible threonine (%)	0.743	0.743	0.743	0.743					

¹Each kg contained: Mn150,000mg, Zn 100,00mg, Fe 100,000mg, Cu 16,000mg, I 1,500mg, Se 300mg, vit. A 8,000,000 IU, vit. D₃ 2,000,000 IU, vit. E 15,000IU, vit. K 1,800mg,vit. B₂ 6,000mg,vit. B₁, 22,800mg,vit. B₁, 12,000mg,niacin 40,000mg,folic acid 1,000mg,pantothenic acid 15,000mg, biotin 60mg, antioxidant 30g.

RESULTS AND DISCUSSION

There was no effect (p>0.05) of DEB values on the performance of broilers in the periods of 7 to 14, 7 to 21, or 22 to 38 days of age, which suggests that broilers were tolerant to DEB changes. These DEB values may influence nutrient absorption in the small intestine, particularly of monosaccharides and amino acids.

Similar results were obtained by Campestrini *et al.* (2008), who studied the effects of grower diets containing 190 and 255 mEq/kg after 22 days of age and did not observe any effect on broiler performance. Szabó *et al.* (2011) evaluated the effects of DEB levels (-50, 25, 100, 175, 250, or 325 mEq/kg) on broiler performance and did not observe any differences in weight gain, feed intake or feed conversion until 14 days of age. The best performance was obtained with 175 mEq/kg and the worse feed conversion ratios

were observed when broilers were fed -50 and 25 mEq/kg, which suggests that broilers may be suffering metabolic acidosis.

Different results were observed by Borges *et al.* (2003b), who observed significant effects of DEB (0, 120, 240 and 360 mEq/kg) on broiler weight gain, feed intake and feed conversion ratio, suggesting that the best DEB values were 186, 176 and 197 mEq/kg for each variable, respectively. Also, Ravindran *et al.* (2008) fed different DEB levels (150, 225, 300 and 375 mEq/kg) to one- to 21-d-old broilers from 1 to 21 days of age and obtained worse weight gain and feed conversion ratio at 375 mEg of DEB.

Considering the results of this experiment, it is possible to observe that broilers are able to adjust their electrolytic balance despite the considerably different DEB values in their diets (from 200 to 320 mEg/kg).

² Each kg contains: Mn 150,000mg, Zn 100,000mg, Fe 100,000mg, Cu 16,000mg, I 1,500mg, Se 250 mg, vit. A 7,500,000 IU,vit. D₃ 1,600,000 IU,vit. E 14,000IU, vit. K 1,700mg, vit. B, 5,800mg, vit. B, 2,500mg, vit. B₅, 2,500mg, vit. B₆, 2,500mg, vit. B₇, 11,000mg, niacin 38,000mg, folic acid 1,000mg, pantothenic acid 14,000mg, biotin 58mg, antioxidant 30g.



Table 2 – Performance of broilers fed diets with different DER values from 7 to 21 and 22 to 38 days of age

DEB values from 7 to 21 and 22 to 38 days of age						
	DEB (mEq/kg)				CV^1	
Parameters	200	240	280	320	(%)	
	7 to 14 days of age					
Body weight (g)	520	490	504	487	4.56	
Weight gain (g)	319	289	306	289	7.56	
Feed intake (g)	412	383	406	404	5.51	
Feed conversion	1.29	1.34	1.33	1.43	3.44	
Mortality rate (%) ²	0.40	0.90	2.60	1.30	8.96	
	7 to 21 days of age					
Body weight (g)	1022	964	1018	1013	4.59	
Weight gain (g)	822	763	819	814	5.69	
Feed intake (g)	1169	1088	1158	1140	3.70	
Feed conversion	1.42	1.43	1.42	1.39	2.09	
Mortality rate (%) ²	3.70	5.40	6.70	5.40	8.96	
	22 to 38 days of age					
Body weight (g)	2452	2423	2482	2410	2.79	
Weight gain (g)	1548	1476	1512	1466	2.25	
Feed intake (g)	2369	2455	2473	2387	3.26	
Feed conversion	1.54	1.66	1.64	1.63	3.50	
Mortality rate (%) ²	0.98	1.47	0.49	0.49	5.84	

¹ CV = coefficient of variation

There was no effect (p>0.05) of DEB on tibial density and mineral content, nor on Na, K and Cl serum levels. However, litter dry matter content was linearly reduced (p>0.001) with increasing DEB levels.

Table 3 – Litter dry matter content, tibialdensity and mineral content, and Na, K and Clserum concentrations of broilers fed increasing DEB levels from 7 to 21 and from 22 to 38 days of age

	DEB (mEq/kg)				CV ¹	
Variables	200	240	280	320	(%)	
	21 days of age					
Litter dry matter content (%) ²	74.41	69.08	72.07	66.11	4.14	
Bone density (g/mL)	1.31	1.33	1.44	1.38	14.13	
Ash (%)	38.58	37.96	38.36	39.63	5.10	
Serum Na+ (mmol/L)	127	1.21	121	123	15.89	
Serum K+ (mmol/L)	5.02	4.85	7.40	5.20	27.74	
Serum Cl ⁻ (mmol/L)	131	137	125	111	19.47	
	38 days of age					
Litter dry matter content (%) ²	58.99	61.52	57.39	49.18	5.17	
Bone density (g/mL)	1.19	1.21	1.23	1.19	6.06	
Ash (%)	41.58	43.29	39.40	41.27	7.18	
Serum Na+ (mmol/L)	120	132	106	134	14.78	
Serum K+ (mmol/L)	3.87	3.72	4.25	3.42	31.92	
Serum Cl ⁻ (mmol/L)	101	109	122	129	21.27	

¹linear effect ($\hat{Y} = 78.20 - 0.0036x, r^2 = 0.61$).

It is important to emphasize that broilers lose 80% of their body heat produced via respiratory system, and significantly increase their water intake during heat stress. Any changes in dietary Na⁺ e K⁺ content also increase water intake and, consequently, litter moisture. Borges (2001) observed that the increase in water intake caused by increasing DEB levels is required to overcome the osmotic imbalance caused by higher Na⁺ and K⁺ levels. Blood osmotic pressure can be used to regulate thirst in broilers.

Borges *et al.* (2003b) evaluated diets with 145 (control), 0, 120, 240 and 360 mEq/kg DEB and also reported that litter moisture linearly increased with DEB values, probably as a response to the higher water intake observed. Water intake and turnover increase as DEB values and broiler age increase, with consequent higher water excretion and wet litter. Ravindran *et al.* (2008) evaluated the effects of DEB levels on excreta dry matter content and observed that the excreta of broilers fed diets with 300 and 375 mEq/kg were more humid (76.9 and 81.2%, respectively) compared with broilers fed 150 and 225 mEq/kg (73.1 and 72.3%, respectively).

These results are consistent with those of Oliveira et al. (2003), who evaluated DEB values between 205 and 325 mEq/kg in broiler diets and verified a linear increase in litter moisture.

Tibia density and mineral content allows estimating if Ca supplementation was adequate. In diets with low acidity (200 mEq/kg) there is no intense Ca mobilization from the bones to excrete metabolic acid blood components through the kidneys. This was demonstrated by Campestrini *et al.* (2008), who fed broilers diets with 190 to 255 mEq/kg DEB and did not find any differences in calcium serum levels at 40 days of age.

Bones function as a buffer system for the electrolyte balance of body fluids. Acid diets can induce cation release (including calcium) from the bones to blood to correct pH. Most calcium in the body is bound to the mineral bone matrix, which suggests that bone is the source to the higher excretion of this mineral by the kidneys (Bushinsky, 2001). Calcium loss should result in reduced bone mineralization and could affect bone density. This was not observed in the present experiment, suggesting that the DEB of 200 mEq/kg did not affect bone metabolism of broilers until 38 days of age.

Moghaddam *et al.* (2005) did not observe any difference in ash content or Na and K levels in the tibias of broilers fed diets with 187, 230, 251 and 284

 $^{^2}$ Mortality (%) for statistical analysis was transformed in Arc Sen= square root (mortality rate/100) + 0.05)).

²linear effect ($\hat{Y} = -31.50 + 0.788x$, $r^2 = 0.99$).



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mEq/kg by increasing CI levels from 103.7 (187 mEq/kg) to 111.4 mmol/L (284 mEq/kg). According to Pitts (1970), CI increase in response to high dietary cation is a mechanism to maintain alkaline blood reserves of blood, which may be sufficient to a possible adjustment of electrolyte balance. Oliveira *et al.* (2010), using the same levels of this experiment (200, 240, 280, or 320 mEq/kg), did not observe any effect on tibial weight, height, diameter, relative weight and weight/length index of 21- to 38-d-old broilers. Evaluating increasing DEB levels (-50, 25, 100, 175, 250, or 325 mEq/kg), Szabó *et al.* (2011) reported that dry matter, ash, and Ca, P, Mg, Na, and K contents of broiler thighbones were not affected.

In conclusion, diets with 200 mEq/kg can be recommended for broilers between seven and 38 days of age to reduce litter moisture without any negative influence on performance, bone density and mineral content.

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