Productive and hematologic responses of country poultry subjected to different housing densities and water salinity levels

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ABSTRACT The aim of this research was to evaluate the production and hematologic responses of confined Rhode Island Red chickens consuming water with 3 different levels of salinity and housed at different densities. Seven hundred and twenty birds were distributed in 36 experimental boxes built inside a poultry house according to a completely randomized design with a 3 × 3 factorial scheme with 3 salinity levels (**SL**) of water (1, 4, and 8 dS/m) and 3 housing densities (8, 10, and 12 birds/m²). Four birds were evaluated from each experimental box, thus totaling 16 repetitions (birds) per treatment. The productive performance, carcass yield, and hematologic traits of the birds in different experimental conditions were evaluated. Increasing water SL resulted in a significant increase ($P < 0.05$) in water and feed consumption beginning in the sixth week of life, causing an increase in the percentage of carcass and heart weight, with no changes in serum responses. Increasing housing density led to a reduction in water and feed consumption, weight gain, and feed conversion, thus reducing the chickens' blood magnesium levels.

Key words: broiler poultry, colonial chicken, poultry confinement, water salinity

INTRODUCTION

Farming country or colonial broilers is a potential activity in Brazil, especially in the northeast region, as they are low-maintenance birds capable of presenting high production results. For efficient husbandry in this region, some factors must be taken into account, such as climatic conditions (Rama Rao et al., 2018), production system (Liu et al., 2011; Fu et al., 2015; Li et al., 2017), and drinking water quality (Al-Mufarrej et al., 2005; El-Sabrout and Hanafy, 2017).

The areas within in this semiarid region experience low annual rainfall and consequently have scarce drinking water (Gomes et al., 2018); the availability of this nutrient is one of the factors that need to be considered when undertaking commercial husbandry of chickens. In addition to low rainfall, another concern to be taken into account is the water quality, as the reservoirs in this region can have water with high electrical conductivities, thus classified as brackish. This is due to the geological characteristics of most of the semiarid soil, specifically the predominance of crystalline rocks in the subsoil (ANA, 2007).

Elevated levels of sodium chloride in drinking water result in increased blood pressure in birds (Honarbakhsh et al., 2007). To expel salt, animals increase water consumption, reducing the amount of potassium in their blood (Kalimuthu et al., 1987; Balnave and Gordon, 1993); consequently, the anion–cation ratio becomes unbalanced. This imbalance can affect many physiological and metabolic bodily functions and, therefore, can reduce the productive performance of animals due an increase in the feed conversion rate (Kalimuthu et al., 1987; Julian, 1993).

The balance of body fluids in animals is meticulously regulated by neuroendocrine control systems (McKinley et al., 2004). After a change in the volume or content of the extracellular fluid (including blood plasma), these control systems provide appropriate compensation to maintain them within narrow ranges. In the body water balance equations, the 2 main variables are water and sodium, with extracellular

osmolarity being regulated mainly by water intake and excretion (Geerling and Loewy, 2008).

Conventional industrial production of broiler chickens is an activity that has high production efficiency due to technological and genetic advances, and Brazil is one of the world's highest producers (ABPA, 2018). High production efficiency has not yet been achieved in the production of free-range chickens. One of the ways through which conventional industrial producers have increased production efficiency is by adopting high densities in bird housing (Rashidi et al., 2018). Thus, to improve efficiency in the production of free-range chickens, it is necessary to define the appropriate housing density (**D**) for these birds in order to ensure adequate conditions so that they can achieve their maximum productive potential (El-Deek and El-Sabrout, 2019).

Therefore, this research aimed to evaluate the production and hematologic responses of Rhode Island Red breed chickens consuming water with increasing levels of salinity and confined to different Ds.

MATERIALS AND METHODS

Experiment Location

The experiment was carried out at the premises of the Federal Institute of Education, Science and Technology of Pernambuco—IFPE, Campus Barreiros, Pernambuco, Brazil. This semiarid region in the northeast of Brazil has a maximum annual precipitation of 800 mm, an average annual temperature ranging from 23°C to 27°C, and an average relative humidity of approximately 50% (Moura et al., 2006).

Animals and Housing

This research was approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil, under Protocol CEP N° 092-2018.

For the following experiments, 720 one-day-old male Rhode Island Red chicks were acquired and were vaccinated for the prevention of Marek's disease, fowl pox, and infectious bronchitis. The experiment was initiated when the birds were 15 d of age, at which point they were weighed individually and marked with plastic rings with different colors fixed on the shank for identification. The experimental period lasted from 23 March to 17 May 2018, totaling 56 d. During the experimental period, the birds were exposed to a light-dark cycle of 23 consecutive hours of illumination with an hour in the dark.

The experimental aviary (Figure 1) was 26.0, 8.2, and 2.8 m in length, width, and height, respectively, oriented in the east-west direction. Within it, 36 experimental boxes with dimensions of 2.0 × 1.1 m were built, with a usable area of 2.0 m^2 (excluding feeder and drinker areas). The boxes were equipped with tubular feeders and bell-shaped drinkers, while the floor was covered with wood shavings, which were replaced whenever necessary.

Experimental Design

After reaching 15 d of age, the birds were distributed in the experimental boxes according to a completely randomized design and a 3 × 3 factorial scheme, with 3 different levels of water salinity and 3 Ds. Four birds were evaluated per experimental box, thus totaling 16 repetitions (birds) per treatment. The tested salinity levels (**SL**) were 1.0, 4.0, and 8.0 dS/m and were adopted based on the results of Luke (1987), which defines 4.7 dS/m as the safe maximum limit of total water salts for chickens. The evaluated densities were 8 (low density), 10 (ideal density), and 12 birds/m^2 (high density), defined based on the guidelines by Schmidt and Figueiredo (2007) for free-range chickens.

Experimental Procedures

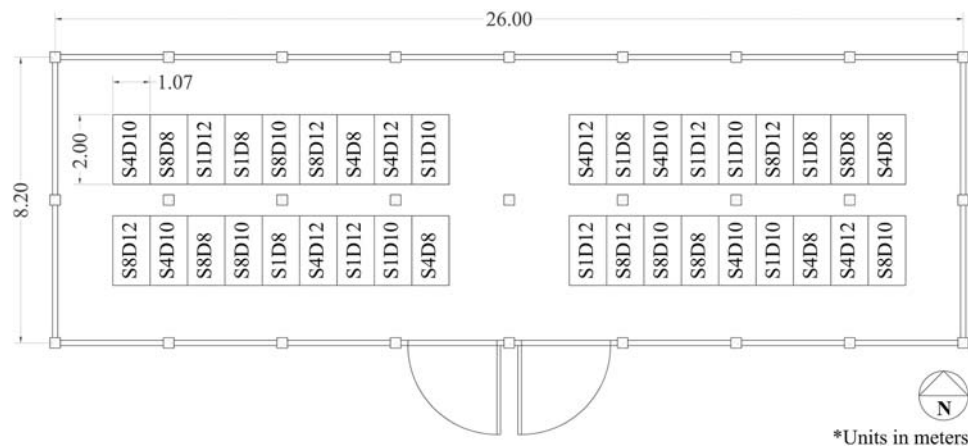Throughout the experimental period, the birds received water and feed ad libitum, and during the first

Figure 1. Floor plan of the shed showing the arrangement of the boxes for each treatment, where S represents the levels of water salinity (1, 4, and 8 dS/m) and D the housing density factor (8, 10, and 12 birds/m^2).

Table 1. Analysis of representative quality parameters of the water offered to chickens.

Parameters analyzed	MAV	Values
Total dissolved solids (mg/L)	500	38.98 ± 2.98
Conductivity (μS/cm)	ND	87.14 ± 0.83
Turbidity (uT)	≤100 uT	32.52 ± 2.17
pH	6.0–9.0	6.21 ± 0.03
Hardness (mg CaCO$_3$/L)	110	65.67 ± 3.07
Chloride (mg Cl$^-$/L)	250	8.75 ± 1.25
Nitrates (mg NO$_3^-$/L)	10	0.60 ± 0.01
Sulfates (mg SO$_4^{2-}$/L)	250	15.30 ± 2.50
Total ammoniacal nitrogen (mg N/L)	13.3	0.29 ± 0.01
Escherichia coli (organisms/100 mL)	Absence	Presence

Abbreviations: MAV, maximum allowed value; ND, undefined; uT, turbidity unit.

week of life, the birds consumed water without additional sodium chloride. The analysis of this water is shown in Table 1, as per the standards established in the Resolution of the National Council for the Environment (CONAMA), Nº. 357 of 17 March 2005. After that, solutions with additional sodium chloride at predetermined concentrations were offered to the chickens.

The diet was formulated based on the NRC (1994) recommendations for broiler birds, which suggests a diet of 3,200 kcal/kg of metabolizable energy and approximately 23, 20, and 18% crude protein in the initial, growth, and final phases, respectively. The formulation is shown in Table 2.

Zootechnical Performance

During the experimentation period, all birds were weighed individually each week. The weekly food and water intake was calculated by measuring the difference between the quantity offered and that left over; subsequently, the total consumption of feed and water per week was calculated by dividing the amount of feed and water consumed by the number of birds in each experimental box, thus determining the average intake per bird. The body weight gain was calculated from the difference between the initial and final weight of the chickens each week. The feed conversion index was calculated by dividing the amount of food consumed in a given period by the weight gain in the same period, expressed in the same unit of weight.

When the chickens were 72 d of age, 4 chickens from each experimental box were selected to determine the carcass yield. The birds were subjected to a 24-hour fast with free access to water, after which they were weighed, stunned, and subsequently slaughtered by cutting the neck. After 5 min of bleeding, each chicken was scalded (60°C for 3 min), plucked, and eviscerated, and the head, neck, and legs were removed. The carcass, without giblets, was weighed and subsequently expressed as a percentage of its live weight (P_{liv}), and this value was considered the carcass yield (P_{carc}). In addition, the weights of the liver (P_{liver}, without gallbladder), gizzard (P_{gizz}), proventricle (P_{pro}), heart (P_{hea}), bursa of Fabricius (P_{fab}), small intestine (P_{small}), blind intestine (P_{blind}), and visible fat (P_{visf}, around the viscera, gizzards, and subcutaneous) were determined, and the relationship between the weight of each organ and the live weight of the birds was calculated as percentages.

Hematologic Responses

From each of the 4 chickens selected in each experimental box, a 10-mL blood sample was collected by brachial vein venipuncture after a 24-hour fast to obtain serum and fluorinated plasma. Both serum and fluoridated plasma were obtained after centrifuging the blood at 3,000 rpm for 10 min. The supernatant was aliquoted in Eppendorf microtubes and stored at −20°C until biochemical analysis.

All serum samples were analyzed in a Labmax 240 automated analyzer (Labtest Diagnóstica S.A., Minas Gerais, Brazil) at the Metabolic and Nutritional Diseases Laboratory of the Advanced Research Center of Goats at the Federal Rural University of Pernambuco (UFRPE).

Table 2. Formulation for 100 kg of poultry feed used.

Macronutrients	Initial ration (kg)	Growth ration (kg)	Final ration (kg)
Ground corn 7.50/730	37	47	57
Ground rice	20	15	10
Soy flakes 45.0/80	38	32	26
Soy oil	2	3	4
Premix CC BC pre-initial 30 kg/T	3	-	-
Premix CC BC growth 30 kg/T	-	3	-
Premix CC BC final 30 kg/T	-	-	3
Total	100	100	100
Nutritional levels			
Metabolizable energy (kcal/kg)	2,988.62	3,117.22	3,235.55
Crude protein (%)	21.46	19.12	16.78
Crude fat (%)	4.20	5.40	6.60
Ash (%)	5.74	5.46	5.24
Total calcium (%)	0.80	0.79	0.66
Total phosphorus (%)	0.45	0.43	0.38
Sodium (%)	0.18	0.19	0.17

Abbreviations: BC, broiler chicken; CC, concentrated core.

Table 3. Productive responses of chickens subjected to different drinking water salinities and housing densities.

Responses	SL (dS/m)			D (birds/m²)			P-value		
	1	4	8	8	10	12	SL × D	SL	D
F1									
Water consumption (mL)	2,384.90 ± 440.69	2,208.96 ± 472.23	2,475.03 ± 518.03	2,803.65 ± 361.36^a	2,291.17 ± 320.30^b	1,972.57 ± 320.24^b	0.938	0.177	0.000
Feed intake (g)	686.67 ± 122.77	712.5 ± 139.08	725.00 ± 113.66	824.17 ± 106.81^a	702.50 ± 87.00^b	597.5 ± 59.75^c	0.544	0.592	0.000
Weight gain (g)	526.37 ± 103.60	554.39 ± 90.90	541.47 ± 92.82	609.10 ± 76.82^a	538.23 ± 86.80^b	474.91 ± 70.58^c	0.092	0.213	0.000
Feed conversion (g/g)	1.32 ± 0.19	1.31 ± 0.15	1.32 ± 0.12	1.36 ± 0.16^a	1.32 ± 0.13^{a,b}	1.28 ± 0.16^b	0.473	0.877	0.028
F2									
Water consumption (mL)	4,697.15 ± 423.05^b	4,896.67 ± 443.60^b	5,157.43 ± 567.44^a	5,420.00 ± 443.14^a	4,904.17 ± 205.90^b	4,427.08 ± 192.86^c	0.137	0.000	0.000
Feed intake (g)	1,844.34 ± 200.30	1,886.07 ± 218.96	1,933.14 ± 234.71	2,130.84 ± 157.08^a	1,863.89 ± 95.18^b	1,668.82 ± 75.98^c	0.651	0.213	0.000
Weight gain (g)	1,061.42 ± 152.51	1,084.33 ± 177.19	1,112.20 ± 151.17	1,156.54 ± 167.00^a	1,085.15 ± 138.66^{a,b}	1,016.27 ± 147.80^b	0.626	0.271	0.000
Feed conversion (g/g)	1.78 ± 0.29	1.77 ± 0.37	1.75 ± 0.26	1.88 ± 0.27^a	1.75 ± 0.24^b	1.67 ± 0.25^b	0.209	0.946	0.001
F3									
Water consumption (mL)	4,015.00 ± 588.54^b	4,198.33 ± 590.16^{a,b}	4,285.50 ± 711.13^a	4,897.50 ± 347.04^a	4,115.00 ± 171.70^b	3,483.33 ± 166.70^c	0.757	0.024	0.000
Feed intake (g)	1,593.71 ± 168.33^b	1,603.25 ± 186.65^{a,b}	1,640.21 ± 198.81^a	1,839.92 ± 63.77^a	1,600.23 ± 28.20^b	1,397.03 ± 27.27^c	0.405	0.024	0.000
Weight gain (g)	733.79 ± 144.76	757.79 ± 187.01	746.06 ± 236.53	806.90 ± 239.85^a	736.19 ± 170.13^{a,b}	694.56 ± 138.70^b	0.590	0.829	0.017
Feed conversion (g/g)	2.25 ± 0.52	2.22 ± 0.52	2.35 ± 0.61	2.44 ± 0.62^a	2.30 ± 0.57^{a,b}	2.08 ± 0.38^b	0.820	0.500	0.006
Total period									
Water consumption (mL)	11,097.05 ± 1,385.02^b	11,303.96 ± 1,395.47^b	11,913.96 ± 1,663.62^a	13,121.15 ± 842.57^a	11,310.83 ± 548.27^b	9,882.99 ± 519.82^c	0.853	0.006	0.000
Feed intake (g)	4,124.73 ± 627.24	4,214.31 ± 541.56	4,285.86 ± 535.57	4,794.93 ± 250.44^a	4,176.62 ± 172.28^b	3,663.35 ± 113.62^c	0.673	0.128	0.000
Weight gain (g)	2,321.58 ± 230.94	2,396.52 ± 450.18	2,399.74 ± 330.71	2,572.53 ± 293.66^a	2,359.56 ± 225.62^{a,b}	2,185.74 ± 200.46^b	0.628	0.209	0.000
Feed conversion (g/g)	1.79 ± 0.19	1.77 ± 0.20	1.80 ± 0.20	1.88 ± 0.21^a	1.78 ± 0.16^b	1.69 ± 0.16^c	0.721	0.720	0.000

[a-c] verages followed by the same letter on the same line do not differ statistically.
The Tukey test was applied at the 5% probability level.
Abbreviations: D, housing density; SL, salinity levels.

Table 4. Effect of drinking water salinity level and housing density on the carcass characteristics of the evaluated chickens.

Responses	SL (dS/m)			D (birds/m²)			P-value		
	1	4	8	8	10	12	SL × D	SL	D
P_{liv} (g)	2,555.92 ± 552.89	2,611.90 ± 391.45	2,635.82 ± 370.67	2,806.53 ± 434.04[a]	2,582.56 ± 369.00[b]	2,411.80 ± 294.96[c]	0.589	0.199	0.000
P_{carc} (%)	69.38 ± 10.76[b]	74.97 ± 7.42[a]	75.73 ± 11.58[a]	72.06 ± 10.39	76.17 ± 11.78	71.85 ± 6.93	0.902	0.004	0.059
P_{liver} (%)	1.37 ± 0.19	1.42 ± 0.29	1.32 ± 0.30	1.32 ± 0.26	1.40 ± 0.28	1.40 ± 0.24	0.927	0.193	0.250
P_{gizz} (%)	2.05 ± 0.42	2.00 ± 0.42	2.06 ± 0.39	1.93 ± 0.41	2.13 ± 0.45	2.04 ± 0.38	0.845	0.795	0.076
P_{pro} (%)	0.36 ± 0.08	0.36 ± 0.07	0.36 ± 0.06	0.35 ± 0.07	0.38 ± 0.06	0.35 ± 0.06	0.593	0.931	0.083
P_{hea} (%)	0.41 ± 0.10[b]	0.46 ± 0.06[a]	0.46 ± 0.07[a]	0.44 ± 0.09	0.43 ± 0.07	0.45 ± 0.06	0.420	0.001	0.853
P_{fab} (%)	0.17 ± 0.10	0.15 ± 0.04	0.15 ± 0.04	0.16 ± 0.10	0.16 ± 0.04	0.15 ± 0.04	0.928	0.343	0.948
P_{small} (%)	3.61 ± 0.61	3.66 ± 0.46	3.63 ± 0.60	3.47 ± 0.56[b]	3.65 ± 0.60[a,b]	3.78 ± 0.47[a]	0.531	0.900	0.022
P_{blind} (%)	1.45 ± 0.43	1.33 ± 0.22	1.4 ± 0.27	1.38 ± 0.24	1.42 ± 0.30	1.38 ± 0.36	0.420	0.160	0.731
P_{visf} (%)	2.48 ± 1.09	2.61 ± 2.26	2.24 ± 0.91	2.56 ± 2.26	2.54 ± 1.02	2.22 ± 1.01	0.256	0.493	0.479

[a-c] Averages followed by the same letter on the same line do not differ statistically.

The Tukey test was applied at the 5% probability level.

Abbreviations: D, housing density; P_{blind}, blind intestine weight; P_{carc}, carcass yield; P_{fab}, bursa of Fabricius weight; P_{gizz}, gizzard weight; P_{hea}, heart weight; P_{liv}, live weight; P_{liver}, liver weight; P_{pro}, proventricle weight; P_{small}, small intestine weight; P_{visf}, visible fat weight; SL, salinity levels.

Statistical Analysis

For optimal data analysis, the experimental period was divided into 3 phases, namely, F1, including the third to the fifth week of life, F2, including the sixth to eighth weeks of life, and F3, including the ninth to 10th week of life. The average measured responses were analyzed when considering the entire experimental period.

The productive and hematologic responses are presented as means ± SEM of the data. For the statistical analysis, the ExpDes.pt package (version 1.1.2, Ferreira et al., 2013, Brazil) of the statistical software R version 3.4.1 (R Core Team, 2013) was used. The residual normality and homogeneity of the sample variances were tested using the Shapiro-Wilk and Bartlett tests, respectively. To evaluate the effects of the different water SLs and Ds on the productive and hematologic responses of the birds, ANOVAs and F tests were used, according to the statistical model presented in Equation 1. The Tukey test was used to compare the averages, with a probability of error of 5% ($P < 0.05$).

$$y_{ijr} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijr} \tag{1}$$

where y_{ijr} is the rth response that received the ith level of factor α and jth level of factor β; μ is the constant (average); α_i is the effect of the ith factor α (water SL), i = 1, 2, 3…a; β_j is the effect of the jth level of factor β (D), j = 1, 2, 3…b; $\alpha\beta_{ij}$ is the interaction effect; and ε_{ijk} is the experimental error.

RESULTS

Productive Responses

The interaction between SLs and D (SL × D) had no significant effect ($P > 0.05$) on any of the productive responses evaluated (Table 3). Notably, when considering the periods from the sixth to the 10th week of life of the birds (F2 and F3), there was a significant difference ($P < 0.05$) in water consumption by the birds as the SLs increased, as it increased 10 and 7%, when comparing the SL from 1 to 8 dS/m in phases F2 and F3, respectively. This was also found when analyzing the total water consumption (considering the entire experimental period), in which there was an increase of approximately 8%.

The chickens consumed a greater ($P < 0.05$) amount of feed with increasing levels of water salinity in the F3 phase. The consumption of water and feed, weight gain, and feed conversion were significantly reduced ($P < 0.05$) with increasing D.

Regarding carcass yield, the effect of the interaction between SLs and D was not significant ($P > 0.05$). Increasing SLs had a significant effect ($P < 0.05$) on the percentages of carcasses and hearts, and as D increased there was a significant increase ($P < 0.05$) in the percentage of the small intestine relative to the live weight (Table 4).

Hematologic Responses

There was no significant interaction ($P > 0.05$) between water SL and D on the hematologic responses of the animals evaluated, which were also not significantly affected ($P > 0.05$) by water SL. The increase in D from 8 to 12 birds/m^2 significantly reduced ($P < 0.05$), by 9.32% on average, the amount of magnesium in the birds' blood, while the remaining blood variables remained unaffected (Table 5).

DISCUSSION

The increase in birds' water consumption with increasing SL can be highlighted as an osmoregulatory tool used to avoid considerable variations in the extracellular fluid sodium concentration via the dynamic balance between water intake and excretion (Geerling and Loewy, 2008).

Increasing water SL caused an increase in chickens' feed intake in the last 2 wk of life, but there was no proportional increase in weight gain. Although there were no relevant changes in feed conversion, one can observe a reduction in the production efficiency of the birds when provided with water with increasing levels of salinity; thus, the chickens had to ingest a larger amount of feed to maintain the same weight gain.

Weight gain and feed conversion were not affected by water SLs, and weight gain was within the average established for the strain. According to Figueiredo et al. (2007), colonial birds with an approximate age of 70 d usually gain approximately 215 g of live weight per week with a feed conversion index of approximately 3.7. It can also be seen that the birds evaluated in the present study showed better feed conversion than the reference values provided by the aforementioned authors, demonstrating the animals' ability to consume saline water without compromising their productive performance (Figueiredo et al., 2007).

The reduction in water and feed intake caused by increasing D may be linked to a lower availability of physical space, thus restricting the birds' mobility and making access to drinking fountains and feeders more difficult (Lima et al., 2018). As a result, the birds' weight gain was also negatively affected by the increase in D, and since the weight gain of chickens under normal density conditions is directly proportional to food intake, it can be inferred that the reduction in weight gain observed in birds evaluated at higher densities is directly related to their lower feed intake.

Similar to what was observed in the present study, Lima et al. (2018) reported a reduction in broiler weight gain as D increased, emphasizing that, despite a significant reduction in weight gain and feed intake, there was still a significant reduction in food conversion; that is, the birds were more efficient in converting metabolic energy from feed into live weight. The authors justified that the decreased feed conversion was due to the reallocation of metabolizable energy that would have been spent on locomotion to weight gain due to the greater

Table 5. Hematologic responses of chickens subjected to different drinking water salinities and housing densities.

Responses	SL (dS/m)			D (birds/m²)			P-value		
	1	4	8	8	10	12	SL × D	SL	D
Magnesium (mg/dL)	2.25 ± 0.27	2.28 ± 0.23	2.22 ± 0.23	2.36 ± 0.26[a]	2.25 ± 0.20[a,b]	2.14 ± 0.21[b]	0.886	0.712	0.027
Phosphorus (mg/dL)	5.65 ± 0.74	5.81 ± 0.69	5.46 ± 0.53	5.54 ± 0.53	5.5 ± 0.61	5.88 ± 0.79	0.986	0.311	0.185
Calcium (mg/dL)	9.58 ± 1.26	9.58 ± 0.80	9.40 ± 0.99	9.65 ± 1.10	9.24 ± 0.91	9.70 ± 1.00	0.744	0.869	0.365
GGT (mg/dL)	27.74 ± 9.5	29.18 ± 7.64	28.88 ± 10.27	30.26 ± 5.30	27.93 ± 7.71	27.61 ± 11.21	0.561	0.871	0.609
Alkaline phosphatase (mg/dL)	1,338.82 ± 538.88	1,542.45 ± 577.76	1,190.67 ± 437.41	1,316.52 ± 675.75	1,361.14 ± 500.41	1,399.28 ± 483.94	0.341	0.152	0.895
Urea (mg/dL)	1.84 ± 1.23	1.74 ± 1.11	1.81 ± 1.17	1.81 ± 1.23	1.38 ± 0.77	2.20 ± 1.29	0.263	0.961	0.099
Albumin (g/dL)	1.31 ± 0.33	1.30 ± 0.14	1.29 ± 0.11	1.30 ± 0.30	1.30 ± 0.13	1.32 ± 0.15	0.057	0.953	0.663
Creatinine (mg/dL)	0.24 ± 0.07	0.23 ± 0.04	0.23 ± 0.06	0.23 ± 0.06	0.23 ± 0.04	0.24 ± 0.05	0.256	0.787	0.632
Total proteins (g/dL)	3.93 ± 0.99	4.06 ± 0.31	3.96 ± 0.41	3.96 ± 0.35	3.93 ± 0.37	4.06 ± 0.45	0.197	0.547	0.621
Sodium (mmol/L)	169.27 ± 40.47	169.11 ± 39.59	169.6 ± 11.25	170.47 ± 168.46	166.23 ± 10.50	171.28 ± 7.25	0.676	0.99	0.319
Potassium (mmol/L)	5.74 ± 1.51	5.79 ± 1.68	5.49 ± 0.83	5.93 ± 1.55	5.25 ± 0.76	5.84 ± 1.07	0.768	0.574	0.059
Total cholesterol (mg/dL)	127.22 ± 15.71	130.71 ± 17.47	124.73 ± 10.44	127.66 ± 32.45	126.69 ± 12.46	128.32 ± 17.93	0.231	0.486	0.947
Triglycerides (mg/dL)	41.89 ± 13.21	49.99 ± 7.80	39.07 ± 4.23	41.08 ± 6.29	41.9 ± 8.45	38.98 ± 7.36	0.856	0.534	0.506
Uric acid (mg/dL)	3.11 ± 1.43	3.18 ± 1.33	3.58 ± 1.41	3.59 ± 1.19	2.85 ± 1.57	3.43 ± 1.27	0.539	0.499	0.204

[a,b] Averages followed by the same letter on the same line do not differ statistically.

The Tukey test was applied at the 5% probability level.

Abbreviations: D, housing density; GGT, gamma glutamyl transferase; SL, salinity levels.

space restriction caused by the increase in D, which makes movement difficult for chickens.

Another important consideration regarding the reduction in feed conversion of birds subjected to higher Ds is that lower feed consumption by these animals may increase their digestive efficiency. In response to dietary restriction, the organism may experience some adjustments, both enzymatic and hormonal. In response to reduced food consumption, an increase in the volumetric capacity of the crop and a decrease in the passage rate are observed (Cherry et al., 1987; May, 1987; Katanbaf et al., 1988). This decrease in the rate of passage and enzymatic adjustments allow for better food digestion and absorption (Macari et al., 1994).

Analyzing the meat production per square meter, it was found to be 21.61, 24.16, and 27.14 kg/m^2 at D values of 8, 10, and 12 birds/m^2, respectively. It can therefore be inferred that even with less weight gain per bird, the density of 12 birds/m^2 provided greater meat production and could be adopted as a way to optimize production costs and use of the facility area. In this way, higher Ds could be used in the production of country poultry, although animal welfare conditions have to be observed.

Increasing SL influenced birds' percentage of carcass weight relative to the live weight, despite not having an effect on the live weight of the animals. This increase can be explained by extracellular fluids retention in the carcass caused by the excess sodium in the birds' diet (Barros et al., 2001). Barlow et al. (1948) found that increased salt levels in the diet of broiler chickens tended to mask the carcass weight data, due to an increase in body water retention. In addition, Barros et al. (2001) reported that the percentage of dry matter in broiler carcasses was not affected by consuming feed with different sodium levels.

The consumption of water with high concentrations of sodium chloride caused an increase in birds' percentage of heart weight relative to the live weight. Frohlich et al. (2018), when evaluating animals consuming diets rich in sodium, observed increased cardiac and left ventricular mass without considerable changes in hemodynamic factors, leading them to suggest that these pathophysiologic changes occurred exclusively due to the impact of sodium. Finally, these authors also state that it is possible that high sodium intake may have direct myocytic effects inducing cardiac hypertrophy. Reports also suggest that factors such as cations (Na$^+$, Ca^{2+}) may be involved in cardiovascular changes (Marban and Koretsune, 1990; Morgan and Baker, 1991).

The present study showed that there was an increase in the weight of the small intestine with an increase in D, a phenomenon that can possibly be explained by an increase in villus height accompanied by an increase in digestive and absorptive function due to the expansion of the surface area of the small intestine that results in increased organ weight (Awad et al., 2009). Reinforcing this idea, we found that feed conversion was reduced

with increased D, showing that the birds converted food into live weight more efficiently in higher D environments.

The increase in D caused a reduction in the birds' blood magnesium levels, which can be explained by the reduction in feed consumption in higher D conditions. Magnesium mainly comes from Premix, and low consumption of these products can cause a deficit in the animals' magnesium level (Severo et al., 2015). In addition, magnesium is a mineral that is reported to reduce stress levels; therefore, animals subjected to high D may have used this mineral to a greater degree.

McDowell (1999) established reference values for the following broiler serum components: phosphorus (3–6 mg/dL), calcium (8.5–19.5 mg/dL), gamma glutamyl transferase (**GGT**) (18–23.4 mg/dL), alkaline phosphatase (1,276–1,506 mg/dL), urea (0–5 mg/dL), albumin (1.6–2 g/dL), creatine (0.1–0.4 mg/dL), total proteins (2.7–5.6 g/dL), sodium (151–161 mmol/L), potassium (4.6–4.7 mmol/L), total cholesterol (125–200 mg/dL), triglycerides (136–166 mg/dL), and uric acid (2.1 and 7 mg/dL). When comparing the serum responses of birds to these reported reference ranges, it can be noted that the hematologic values, except for GGT, albumin, potassium, and triglycerides, are within the normal range, and none of the assessed responses demonstrated significant changes ($P > 0.05$) as a result of the evaluated treatments.

GGT levels in the evaluated birds were approximately 18% higher than the reported maximum limit, and this increase may be associated with liver damage suffered by the birds. This was mainly caused by the high intake of energy from the feed due to the intensive rearing system (Traesel et al., 2011), which resulted in fat accumulation in the liver. That in turn can cause progressive organ damage in the form of infiltrations and, consequently, increase serum GGT levels (Angulo et al., 1999).

In comparison with the limits proposed by McDowell (1999), the levels of albumin and triglycerides in the birds were 18 and 69%, respectively, below the normal rates for broilers. The birds' deficiency of blood albumin and triglycerides corroborates the hypothesis that they have suffered some type of liver damage, as these proteins are synthesized by the liver (Traesel et al., 2011). As the evaluated chickens were fed diets with low levels of lipids that are digested to form triglycerides, the liver plays a fundamental role in providing these lipids (Hermier, 1997), a process which was deficient in the birds evaluated in this research.

This research shows that free-range Rhode Island Red chickens have a high degree of adaptability to consume water with a high SL, with no significant changes ($P > 0.05$) in their serum markers. Although the adoption of a high D (12 birds/m^2) caused a reduction in feed consumption and weight gain in the animals, this proved, in productive terms, to be the best density of those evaluated, as it resulted in 6 and 3 kg more meat production per square meter compared with the densities of 8 and 10 birds/m^2, respectively.

CONCLUSION

An increase in SLs causes an increase in birds' water consumption beginning in the sixth week of life and feed consumption beginning in the ninth week, but does not affect younger birds. This also caused an increase in the percentages of carcasses and heart weight relative to the live weight of birds. The adoption of a D of 12 birds/m^2 (high density) causes a reduction in water consumption, feed intake, weight gain, and feed conversion in Rhode Island Red chickens. This consequently causes a reduction in birds' body magnesium levels and an increase in the percentage of small intestine weight relative to the animals' live weights.

DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication.

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