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ORIGINAL ARTICLE

Effects of sodium intake through drinking water on muscle hydration and hematological parameters in broiler chickens

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ABSTRACT. This study evaluated the effect of sodium chloride (NaCl) administered through drinking water on muscle moisture content and associated hematological responses in broiler chickens (Cobb 500). Five experimental groups were evaluated, including a control group and drinking-water supplementation with sodium chloride (NaCl) or sodium bicarbonate ($NaHCO_3$) administered at different concentrations and exposure periods. Moisture content of the internal *rectus femoris* muscle was quantified, and hematological parameters were analyzed to evaluate systemic responses related to tissue hydration. Increased muscle moisture content was associated with alterations in leukocyte profiles, including changes in neutrophils, leukocytes, monocytes, and lymphocytes, suggesting changes in circulating immune cell distribution rather than a direct suppression of immune function. Both supplementation regimens produced comparable hematological patterns, and no statistically significant differences were observed between the 2.5 g/L NaCl for 30 days and 5 g/L NaCl for 10 days treatments. Hematological variables were analyzed using an automated hematology analyzer, and statistical analyses included descriptive statistics, permutational multivariate analysis of variance, and principal component analysis, following verification of variance homogeneity and collinearity. The results demonstrate that sodium chloride intake through drinking water is associated with increased muscle moisture content accompanied by measurable hematological changes in broiler chickens. These findings suggest that broilers may serve as a useful experimental model for studying salt-induced alterations in muscle hydration and immune-related parameters under controlled production conditions.

Keywords: sodium load, tissue hydration, leukocyte profile, osmotic stress, avian model.

INTRODUCTION

Edema, clinically recognized as visible or palpable swelling, results from the abnormal accumulation of interstitial fluid caused by disruptions in capillary filtration forces or impaired lymphatic drainage (Smith *et al*., 2024). From a physiological perspective, edema reflects an imbalance between osmotic forces, vascular permeability, and interstitial fluid handling. Although edema is commonly associated with conditions such as heart failure, hepatic disease, and acute inflammation, its early development and potential association with dietary factors, particularly excessive intake of common salt (NaCl), remain insufficiently understood (Stücker *et al*., 2023).

Sodium plays a central role in extracellular volume regulation. Excess Na^+ increases the osmotic pressure of the interstitial compartment and promotes water retention, especially when venous return or lymphatic function is compromised (SEER Training, n.d.). Tissues with high osmotic storage capacity, such as skeletal muscles, may undergo rapid increases in water content, a subtle physiological change that may precede clinically detectable peripheral edema (Frigeri *et al*., 2004).

At the systemic level, high salt intake has been linked to hypertension and endothelial dysfunction, involving immune activation, increased production of reactive oxygen species, and microvascular alterations (Oparil *et al*., 2018). Although dietary salt alone may not directly trigger edema, it can exacerbate pre-existing vascular abnormalities and enhance vascular permeability, facilitating fluid extravasation into peripheral tissues, such as the lower limbs (Borrelli *et al*., 2020). Collectively, these processes suggest that salt-induced fluid retention may not only alter tissue hydration but also modulate the distribution and function of immune cells.

Human studies on salt-related edema are largely restricted to clinical observation and seldom allow continuous assessment of microvascular or tissue-level changes induced by sodium intake. Consequently, animal models offer a valuable opportunity to examine the early changes in tissue hydration and fluid balance, and their mechanistic relationship with salt consumption (Mukherjee *et al*., 2022; Watanabe *et al*., 2025). Such models are particularly useful in species characterized by rapid growth rates and high sensitivity to osmotic and metabolic challenges.

In this context, broiler chickens (*Gallus gallus domesticus*) represent an alternative and underexplored model. This species exhibits marked sensitivity to osmotic imbalance, rapid growth, controlled water intake, and allows for the direct measurement of peripheral parameters, such as thigh circumference and muscle hydration. Despite these advantages, the relationship between high salt intake and muscle hydration in broiler chickens has not been previously investigated. To our knowledge, no previous studies have experimentally evaluated the combined effects of sodium exposure patterns on muscle hydration and hematological responses in broiler chickens

Therefore, this study aimed to evaluate the effects of distinct sodium intake patterns, including a low-dose long-term regimen and a high-dose short-term regimen, on thigh muscle water content and hematological parameters in broiler chickens. By integrating tissue-level and systemic responses, this study seeks to provide insights into the temporal dynamics of sodium-induced fluid redistribution and its association with early alterations in tissue fluid balance and immune-related parameters.

MATERIALS AND METHODS

Study design

The study was conducted at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara, Mexico (20º25′30″–20º57′00″ N; 103º19′30″–103º39′20″ W; 1,548 m a.s.l.). The region has a mean annual temperature of 22 °C (range: 11–36.1 °C) and an average annual precipitation of 906.1 mm (García, 2004). A completely randomized experimental design was employed. Five drinking water treatments were evaluated: 1) control (deep-well water without salt supplementation), 2) sodium chloride (NaCl) at 2.5 g/L for 30 d, 3) NaCl at 5 g/L for 10 d, 4) sodium bicarbonate ($NaHCO_3$) at 10 g/L for 10 d, and 5) $NaHCO_3$ at 5 g/L for 30 d. All treatments were supplied *ad libitum*. Water intake was recorded throughout the experimental period and subsequently analyzed using one-way analysis of variance (ANOVA) to evaluate the potential differences among the treatments. All experimental groups received the same basal diet throughout the study, ensuring that the dietary sodium intake remained constant across the treatments. The selected NaCl and $NaHCO_3$ concentrations were chosen to generate a gradient of sodium exposure, allowing the evaluation of dose-dependent effects under physiologically relevant conditions, based on ranges reported in poultry production and experimental studies evaluating electrolyte balance and osmotic stress in broilers. These levels were designed to represent both moderate and elevated sodium intake scenarios without compromising animal viability. The experimental unit was the individual bird. A total of 100 male Cobb500 broiler chickens were randomly allocated to the five treatments (n = 20 birds per treatment) after body weight homogenization on day 22. All experimental procedures were conducted in accordance with institutional and national guidelines for animal care and use and were approved by the Institutional Bioethics Committee of CUCBA, Universidad de Guadalajara (Approval No. CINV-C/011/2026).

Experimental animals and husbandry

One hundred one-day-old male Cobb500 broiler chicks were obtained from a commercial hatchery and housed under standard broiler management conditions (Cobb-Vantress, 2023). During the brooding period, the birds were maintained in a galvanized wire mesh pen (1.30 × 2.75 m) with rice husk litter. The ambient temperature was initially set at 33 °C and gradually reduced until the brooder heaters were removed on day 20.

Birds were fed a commercial starter diet from arrival until day 22, followed by a gradual transition to finisher diets formulated according to Cobb500 nutritional guidelines and adjusted to meet 95% of the National Research Council requirements (NRC, 1994). The diet corresponded to a complete commercial feed formulated to meet the standard nutritional requirements for broiler chickens and included a vitamin–mineral premix (commonly referred to as a “nutra” component in field terminology). The estimated sodium (Na) content of the basal diet was approximately 0.15% (150 mg per 100 g of food), which is consistent with typical industrial formulations. The dietary composition remained constant across treatments, ensuring that drinking water supplementation was the primary experimental variable. The ingredient compositions and calculated nutrient contents of the experimental finisher diets are presented in Table 1.

Table 1.
Ingredients and calculated nutrient composition (%) of the experimental finisher diets for broiler chickens.

Ingredient	T1	T2	T3	T4	T5
Sorghum	62.000	62.000	62.000	62.000	62.000
Soybean meal 46 %	27.204	25.522	26.495	29.241	27.116
Alfalfa meal 19 %	3.736	3.650	3.776	2.090	3.313
Soybean oil	4.390	4.432	4.360	3.303	4.121
Calcium carbonate 38 %	1.500	2.100	1.190	1.800	1.648
Phosphorus 18/20	1.005	2.000	1.600	1.000	1.401
Lysine HCl	0.037	0.110	0.250	0.361	0.190
DL-Methionine 99 %	0.113	0.137	0.300	0.200	0.188
Threonine	0.015	0.050	0.027	0.000	0.023
Nutrient					
Metabolizable energy (Mcal/kg)	3.05	3.08	3.05	3.05	3.058
Crude protein (%)	20	20	20	20	20
Total calcium (%)	0.9	1.0	0.9	1.0	0.95
Total phosphorus (%)	0.5	0.4	0.4	0.4	0.425
Lysine (%)	1.0	1.0	1.146	1.161	1.077
Methionine (%)	0.4	0.4	0.581	0.497	0.470
Tryptophan (%)	0.18	0.231	0.244	0.254	0.227
Threonine (%)	0.74	0.74	0.74	0.757	0.744
Fiber (%)	3.5	3.8	3.5	2.62	3.355

Diets formulated following NRC (1994) requirements. Finisher diet: 20% CP, 3000 kcal/kg ME. Nutrient estimations calculated with NUTRION 10®. Sodium content was not included in the calculated nutrient profile but was estimated at approximately 0.18% based on manufacturer formulation data.

Lighting schedules followed standard broiler recommendations: 24 h light (days 1–3), 23L:1D (days 4–7), 21L:3D (days 8–15), and 19L:5D (days 16–21), followed by a stable dark period thereafter. The stocking density, ventilation, and litter conditions were maintained within the recommended welfare standards.

All diets were formulated to meet the nutritional requirements of broiler chickens according to the NRC (1994) recommendations and Cobb500 management guidelines. The vaccination program was included as part of the standard husbandry practices and was applied uniformly across all experimental groups, thereby minimizing its potential confounding effect on hematological outcomes. Although vaccination may transiently influence immune parameters, its consistent application allows for valid comparative interpretation among treatments.

Inclusion and exclusion criteria

Only clinically healthy birds were included at baseline. Birds showing signs of parasitic infection, marked weakness, or abnormal growth were excluded prior to treatment allocation. During the experimental period, the birds were monitored daily for health status and welfare. Humane endpoints were predefined and included severe illness, moribund condition, or marked body weight loss. No birds met the exclusion criteria during the study, and all the allocated birds were included in the final analyses. A standard regional vaccination program appropriate for broiler production was uniformly applied across all the experimental groups throughout the study. The complete vaccination schedule is detailed in Table 2.

Table 2.
Vaccination schedule applied during the experimental period.

Day	Vaccine	Route
1	Marek	In ovo
1	Fowl pox (hatchery)	—
7	Gumboro	Ocular
10	Newcastle disease (LaSota strain)	Ocular
20	Avian influenza H5N2, H7N1	Subcutaneous
20	Newcastle disease	Subcutaneous
20	Gumboro	Ocular
33	Newcastle disease (LaSota, lyophilized)	Ocular

Randomization and blinding

Random allocation to treatments was performed on day 22 using a simple randomization procedure after body weight homogenization to ensure a balanced group distribution. Cage placement within the experimental room was periodically rotated to minimize potential environmental confounders related to airflow, light exposure, or localized temperature gradients. Personnel responsible for daily animal care were aware of the treatment allocation to ensure the correct administration of drinking water treatments. Laboratory analyses and statistical evaluations were conducted by investigators blinded to the treatment identity to reduce measurement and interpretation bias.

Sample collection and assessment of muscle hydration

At 58 days of age, the birds were humanely slaughtered according to institutional guidelines. Blood samples were collected immediately prior to slaughter from the clavicular vein using heparinized Vacutainer tubes for hematological analysis. Following slaughter, thigh samples of the internal *rectus femoris* muscle were excised. The muscle moisture content was determined gravimetrically. Samples were weighed fresh and then dried in a forced-air oven at 105 °C for 36 h until a constant weight was achieved. Moisture percentage was calculated as the difference between fresh and dry weights. This method provides a quantitative estimate of muscle water content and was used in this study as an indicator of tissue hydration status. It does not directly assess edema in the strict pathological sense, but rather reflects changes in intramuscular fluid content associated with sodium intake.

Hematological analysis

Hematological parameters were analyzed using a MISTIQ18 veterinary hematology analyzer (70 µL whole blood per sample). The analyzer quantified leukocytes, erythrocytes, platelets, and hemoglobin levels using electrical impedance and colorimetric methods. Differential leukocyte counts, including lymphocytes, monocytes, and granulocytes, were expressed as both absolute values and relative percentages.

Statistical analysis

Data were analyzed using R version 4.1.1 (R Core Team, 2021). Muscle moisture and hematological variables were summarized descriptively and expressed as mean ± standard deviation (SD). Prior to the multivariate analysis, collinearity among the variables was evaluated using Spearman's correlation coefficients.

Treatment effects were assessed using permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distance matrices with 999 permutations, implemented using the *adonis2* function of the vegan package (Oksanen *et al*., 2022). Homogeneity of multivariate dispersion was evaluated using *betadisper*. When significant global effects were detected, pairwise comparisons were performed using *pairwiseAdonis*.

Principal component analysis (PCA) was used to explore multivariate patterns among muscle moisture and hematological variables and was applied only to treatment groups showing significant contrasts in PERMANOVA to facilitate the interpretation of biologically relevant variation.

Ethical review

All animal procedures were performed in accordance with the institutional guidelines for the care and use of laboratory animals at the Universidad de Guadalajara. The study protocol was reviewed and approved by the Bioethics Committee of CUCBA, which functions as the institutional animal care and use committee. Humane care and use standards were followed throughout the study, and the animals were monitored daily to prevent unnecessary distress. Humane endpoints were established to safeguard animal welfare, including the presence of parasitic infections, moribund conditions, or marked body weight loss.

RESULTS

Muscle moisture content

Administration of sodium chloride (NaCl) or sodium bicarbonate ($NaHCO_3$) through drinking water resulted in measurable differences in gravimetric muscle moisture values of the internal *rectus femoris* muscle in Cobb500 broiler chickens (Figure 1).

Control birds exhibited the lowest muscle moisture values. Broilers receiving NaCl supplementation showed higher values of muscle moisture content. The group receiving 2.5 g/L NaCl for 30 d exhibited an increase of approximately 66% relative to the control group, whereas birds exposed to 5 g/L NaCl for 10 d showed the highest muscle moisture values, with an increase of approximately 90%.

$NaHCO_3$ treatments also increased muscle moisture content, although to a lesser extent than NaCl. Birds receiving 10 g/L $NaHCO_3$ for 10 d showed an approximate 25% increase compared with controls, while those receiving 5 g/L $NaHCO_3$ for 30 d showed an increase of nearly 50%. Overall, the NaCl-treated groups displayed greater muscle hydration than the $NaHCO_3$-treated groups (Figure 1).

Water intake

Water intake data are presented in Table 3. No statistically significant differences were observed among the treatments (one-way ANOVA, $P = 0.927$). Mean water consumption values were comparable across all groups, with no consistent pattern associated with sodium supplementation. These findings indicate that the differences in sodium exposure among the treatments were primarily attributable to the composition of drinking water rather than variations in water intake.

Table 3.
Water intake in broiler chickens across experimental treatments.

Treatment	Mean water intake (mL/day)	Standard deviation (±)
Control	504.8	64.3
$NaHCO_3$ 10 g/L (10 d)	517.9	79.6
$NaHCO_3$ 5 g/L (30 d)	501.4	76.6
NaCl 2.5 g/L (30 d)	497.5	71.6
NaCl 5 g/L (10 d)	529.3	61.2

Water intake recorded in broiler chickens across experimental treatments. Values are expressed as mean daily consumption per bird ± standard deviation. No statistically significant differences were observed among treatments ($P > 0.05$).

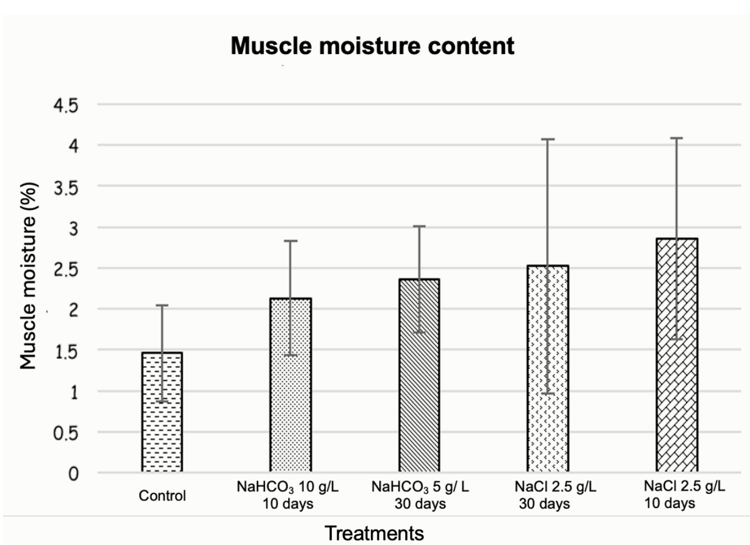

Figure 1.
Muscle moisture content (%) in the internal *rectus femoris* muscle of broiler chickens exposed to sodium supplementation through drinking water. Moisture was calculated as the percentage of weight loss after drying relative to wet muscle weight. Values are presented as mean ± SD.

Hematological parameters

Nineteen hematological variables were evaluated and expressed as absolute counts and relative percentages. Descriptive summaries of leukocyte profiles are presented in Figures 2A and 2B, showing the mean ± standard deviation for absolute leukocyte counts and relative leukocyte proportions across experimental groups. These data are presented to illustrate the distribution and variability of the hematological parameters among treatments. NaCl supplementation was associated with lower absolute counts of monocytes and granulocytes compared with the control group. Both NaCl treatments also showed reduced absolute lymphocyte counts. In contrast, the $NaHCO_3$-treated groups exhibited greater interindividual variability, with hematological patterns partially overlapping with those observed in NaCl-treated birds. Analysis of relative leukocyte distributions revealed distinct patterns among treatments. Birds receiving NaCl exhibited higher relative lymphocyte percentages accompanied by lower monocyte and granulocyte proportions. Sodium bicarbonate treatments showed wider dispersion in relative leukocyte percentages, indicating less consistent effects across individuals (Figure 2B).

Multivariate analysis

Permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distance matrices detected significant overall differences among treatments (F = 1.493, P = 0.037). The assumption of homogeneity of multivariate dispersion was met (F = 0.134, P = 0.9685), supporting the validity of the multivariate comparisons.

Pairwise PERMANOVA comparisons revealed significant differences between the control group and the 2.5 g/L NaCl (30 d) treatment (P = 0.006), as well as between the control group and the 5 g/L NaCl (10 d) treatment (P = 0.015). No statistically significant differences were detected among the remaining treatment comparisons. Principal component analysis (PCA) was subsequently performed using only the treatment groups that showed significant multivariate contrasts (control, 2.5 g/L NaCl, and 5 g/L NaCl). The first two principal components explained 86.1% of the total variance (PC1 = 62.9%; PC2 = 23.2%) (Figure 3).

PC1 was positively associated with muscle moisture content and lymphocyte percentage (PLIN), and negatively associated with monocyte (PMON) and granulocyte (PGRAN) percentages. PC2 was primarily associated with total leukocyte percentage (PLEU). The PCA score plot showed a clear separation between NaCl-treated birds and controls along PC1, reflecting coordinated changes in muscle hydration and leukocyte distribution.

DISCUSSION

The present study demonstrated that sodium intake through drinking water influences muscle water content and is associated with changes in the hematological parameters of broiler chickens. Although the osmotic effects of sodium on fluid retention are well established, the present study provides additional insights by demonstrating how different exposure patterns (chronic low-dose vs. acute high-dose intake) influence tissue-level water distribution and are accompanied by distinct hematological responses in a rapidly growing animal model.

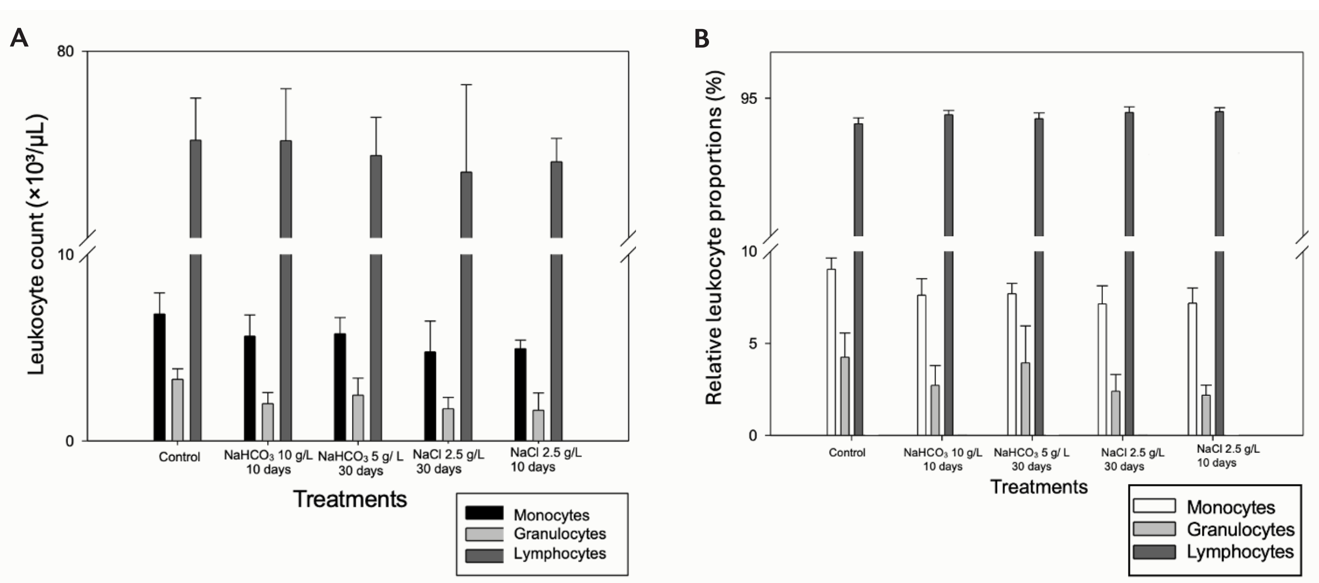

Figure 2.
(A) Absolute leukocyte counts in broiler chickens exposed to sodium supplementation through drinking water. (B) Relative leukocyte proportions (%) of lymphocytes, monocytes, and granulocytes among experimental groups. Values are expressed as mean ± SD.

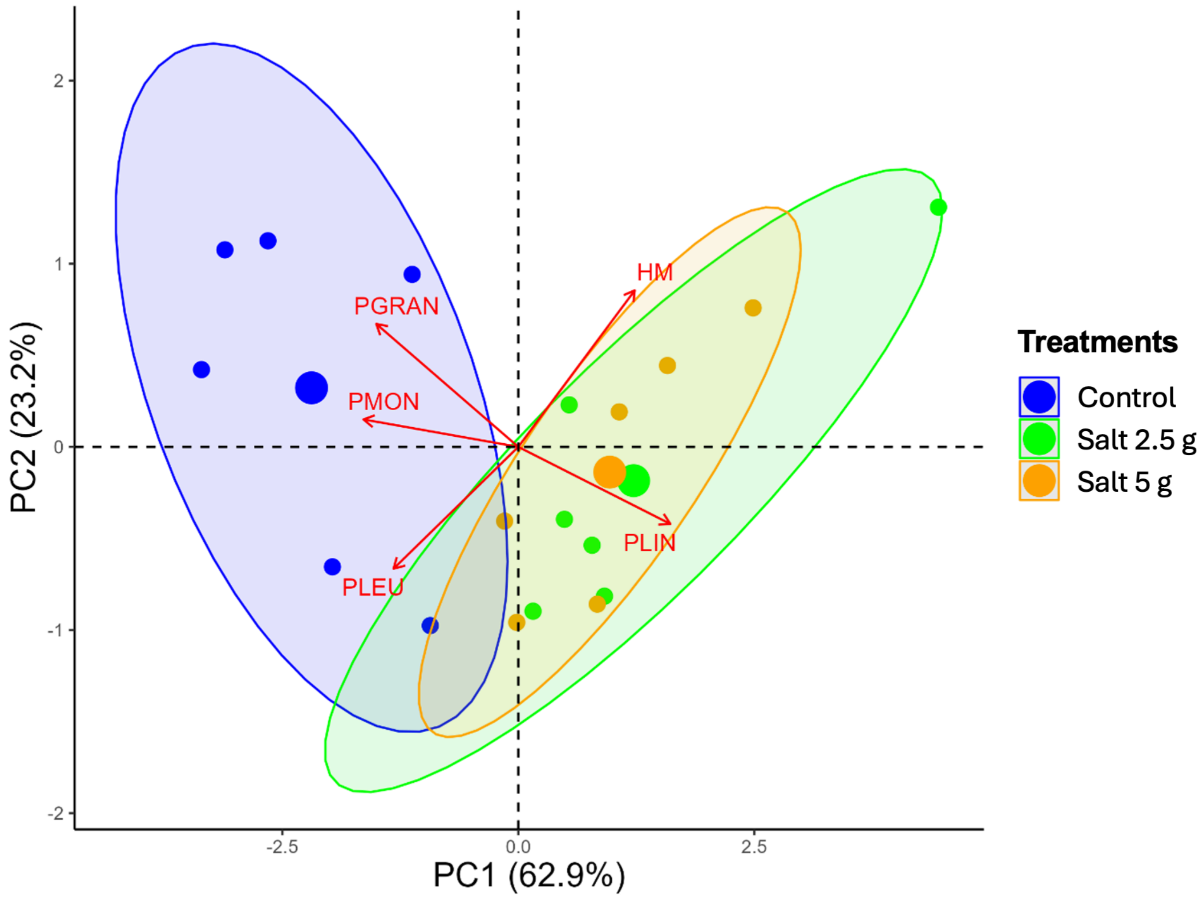

Figure 3.
Principal component analysis (PCA) of muscle moisture and hematological profile variables in broiler chickens from control and salt-treated groups. Each point represents an individual bird, and colors indicate experimental treatments. The first and second principal components (PC1 and PC2) are shown on the x- and y-axes, respectively, with the percentage of explained variance indicated in parentheses. Red arrows represent variable loadings and indicate the direction and contribution of each variable to the PCA ordination. Ellipses represent the 95% confidence ellipses for each treatment group.

Electrolytes play a fundamental role in maintaining cellular electroneutrality and enabling nerve and muscle excitability, with sodium and bicarbonate being among the most physiologically relevant ions supplied through diet and drinking water (Shrimanker & Bhattarai, 2023). Although renal mechanisms are central to systemic electrolyte homeostasis, accumulating evidence indicates that sodium can be stored non-osmotically in peripheral tissues, particularly in the skin and skeletal muscle. Such tissue-level sodium retention has been linked to osmotic imbalance, endothelial dysfunction, inflammation, and the development of chronic disease (Alsouqi *et al*., 2022; Fang *et al*., 2023; Sulyok *et al*., 2022).

It is important to note that the assessment performed in this study, based on a gravimetric approach, reflects muscle water content rather than edema in the strict pathological sense. Although increased water content is consistent with fluid accumulation at the tissue level, it does not by itself demonstrate interstitial fluid imbalance or clinically defined edema. Therefore, the term "muscle hydration" is used to describe the observed changes, which may represent early or subclinical alterations in tissue fluid balance associated with sodium intake. This distinction is particularly relevant when interpreting findings within a physiological rather than a pathological framework.

Local sodium accumulation increases interstitial osmotic pressure, thereby promoting water movement into the extracellular compartment and contributing to alterations in tissue fluid balance.

Broiler skeletal muscle is particularly sensitive to osmotic variation due to its high water content, rapid glycolytic metabolism, and well-characterized electrolyte transport mechanisms. In addition, broiler chickens have been widely used to study fluid balance, endothelial function, and edema-related pathophysiology (Wideman, 2016; Olkowski, 2018), supporting their suitability as an experimental model for sodium-driven alterations in tissue hydration. In the present study, NaCl administration consistently increased moisture content in the m. *rectus femoris*, with short-term high-dose exposure producing the most pronounced effects. Importantly, lower-dose but longer-duration NaCl treatments also induced substantial intramuscular fluid accumulation, indicating that both sodium concentration and exposure duration contribute to fluid accumulation at the tissue level, which may represent the early stages of fluid balance alterations. Notably, the group receiving 2.5 g/L NaCl exhibited greater variability in muscle hydration values compared to other treatments. This variability may be explained by differences in individual water intake, which directly influence sodium exposure under drinking water supplementation. Additionally, physiological heterogeneity among birds, including differences in growth rate, metabolic demand, and fluid regulation capacity, may have contributed to the observed dispersion. It is also possible that the intermediate sodium concentration used in this treatment induced a less uniform physiological response, in contrast to higher concentrations that may trigger more consistent osmotic adjustments. These factors should be considered when interpreting the variability observed in this group. These findings are consistent with established mechanisms, whereby elevated extracellular Na^+ increases local osmolarity and drives water retention within interstitial compartments (Olde Engberink *et al*., 2020; Wiig & Swartz, 2012). In addition, the interval between treatment cessation and slaughter in short-term treatments may have influenced the persistence of the observed effects and should be considered when interpreting these results.

Under physiological conditions, interstitial fluid accumulation is counterbalanced by lymphatic drainage, which returns excess extracellular fluid to the circulation. However, sustained sodium loading may overwhelm the lymphatic transport capacity, particularly in metabolically active tissues, such as skeletal muscles, where osmotic gradients can fluctuate rapidly. Experimental studies have shown that high sodium intake can impair lymphatic pumping and contractility (Scallan *et al*., 2016), providing a plausible physiological explanation for the muscle hydration observed in NaCl-treated birds in the present study.

In addition to its effects on tissue hydration, sodium intake was associated with alterations in hematological parameters. Absolute counts of lymphocytes, monocytes, and granulocytes were reduced in the NaCl-treated groups, whereas proportional analyses revealed a relative increase in lymphocyte percentages accompanied by a reduction in myeloid cell proportions. This pattern reflects an overall reduction in circulating leukocyte populations rather than a selective expansion of specific immune subsets. Such changes are consistent with systemic responses to osmotic stress, endothelial activation, and altered nitric oxide signaling, which are recognized consequences of excessive sodium intake (Afsar *et al*., 2018; Flores & Nugent, 2025; Wang *et al*., 2025). Comparable leukocyte redistribution patterns have been reported in cardiovascular and metabolic disorders associated with high-salt states and vascular remodeling (Flores *et al*., 2025; Mihalj *et al*., 2024), underscoring the translational relevance of evaluating sodium-associated changes in leukocyte profiles in controlled animal models.

Multivariate analyses further supported the integrated nature of these physiological responses. PERMANOVA identified significant treatment-related differences, and principal component analysis clearly separated NaCl-treated birds from control animals along the primary axis of variation. This axis was positively associated with muscle moisture content and lymphocyte proportion and negatively associated with monocyte and granulocyte proportions, indicating that sodium intake induces coordinated physiological and hematological responses rather than isolated parameter changes. In contrast, $NaHCO_3$ treatments did not produce comparable multivariate separation, suggesting that the observed effects are more closely linked to chloride-dependent sodium loading than to generalized electrolyte supplementation (Sadki *et al*., 2026). In addition to NaCl, $NaHCO_3$ treatments were included to explore whether the observed responses were driven solely by the sodium load or were also influenced by the accompanying anion. While both salts provide equivalent sodium, their physiological effects may differ because of the role of chloride in osmotic balance and fluid retention, as well as the involvement of bicarbonate in acid-base regulation. These differences may contribute to distinct patterns of tissue hydration and hematological responses, suggesting that not all sodium sources exert identical biological effects (Baliga *et al*., 2026).

Several limitations of this study should be acknowledged. The relatively small sample size, which is inherent to exploratory physiological studies in poultry, may limit the detection of more subtle treatment effects. Water intake was recorded but did not differ significantly among the groups; however, qualitative observations suggested temporal variations in drinking behavior, with some birds consuming more water during specific periods of the day. These patterns were not quantitatively assessed and may have influenced short-term sodium exposure. Future studies incorporating higher temporal resolution in water intake measurements are warranted. In addition, direct functional assessments of lymphatic transport were not performed. Future studies incorporating lymphatic imaging techniques or molecular markers of lymphatic function would help further clarify the mechanisms underlying sodium-induced muscle hydration. In addition, direct functional assessments of lymphatic transport were not performed. Future studies incorporating lymphatic imaging techniques or molecular markers of lymphatic function would help further clarify the mechanisms underlying sodium-induced muscle hydration.

Taken together, these findings demonstrate that NaCl intake was associated with early and measurable changes in muscle hydration, accompanied by hematological alterations consistent with changes in leukocyte profiles associated with excess sodium. Although human studies have linked high sodium intake to hypertension, cardiovascular disease, and immune dysregulation affecting large populations worldwide (Wang *et al*., 2020), such studies are often limited by heterogeneous dietary patterns and environmental confounders. The controlled avian model employed in this study allows the isolation of sodium-specific effects on tissue hydration and peripheral immune composition under standardized conditions. Although important physiological differences exist between avian and human systems, this model provides a useful experimental platform for exploring the early mechanisms associated with sodium-induced alterations in fluid balance and immune responses. Accordingly, Cobb500 broiler chickens may serve as a complementary model for investigating sodium-related physiological processes, with potential relevance to both veterinary and comparative biomedical research.

It is important to note that the birds in this study were evaluated at 58 days of age, representing a later growth stage than that typically used in commercial broiler production systems. At this stage, increased body mass and metabolic demand may influence fluid regulation and sodium handling. Therefore, the extrapolation of these findings to younger birds should be made with caution, and further studies across different growth stages are warranted.

A limitation of the present study is that sodium concentration and duration of exposure were not independently controlled, which prevents a strict differentiation between dose-dependent and time-dependent effects. However, this design was intended to explore the combined impact of sodium load and exposure period under conditions that may resemble practical production scenarios. Future studies using fully balanced dose-response designs with standardized exposure times are warranted to further elucidate the specific contribution of each factor. Additionally, the total sodium intake from feed and water was not independently quantified, which limits the precise estimation of overall sodium exposure. These limitations should be considered when interpreting the results and highlight the need for further controlled studies.

DECLARATIONS

Competing interests statement

The authors declare that they have no competing interests that could directly or indirectly influence the content or interpretation of this manuscript.

Ethics statement

This study was conducted in accordance with the institutional guidelines and approved by the corresponding ethics committee (Approval No. CINV-C/011/2026).

Data availability statement

All datasets generated and analyzed during this study are maintained in our laboratory at the University of Guadalajara and are available from the corresponding author upon reasonable request for purposes of verification or future research.

Author contributions

Conceptualization: R.R.-O., B.M., Y.R.-Y.; Data curation: R.R.-O.; Formal analysis: R.R.-O., C.J.-P.; Investigation: M.G.L.-R., I.Y.C.-M., B.M.; Methodology: B.M., C.J.-P.; Resources: S.M.-F.; Supervision: Y.R.-Y.; Writing-original draft preparation: R.R.-O.; Writing-review and editing: all authors. All authors have read and agreed to the published version of the manuscript

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