

## Effects of Dietary Sodium Levels on Growth Performance, Hematological Indices, and Tibial Development in Broiler Chickens: Postprint

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### Abstract

This experiment aimed to investigate the effects of dietary sodium levels on growth performance, blood ion concentrations, hematological parameters, blood gas, and tibia indices in broiler chickens. A total of 270 one-day-old Arbor Acres (AA) male broiler chicks were selected and randomly divided into 3 groups (6 replicates per group, 15 birds per replicate), and fed diets with chloride meeting requirements but sodium levels of 0.075% (low sodium group), 0.160% (medium sodium group), and 0.245% (high sodium group). The experimental period was 42 days, divided into three phases: starter (1-14 days), grower (15-28 days), and finisher (29-42 days). The results showed: 1) With increasing dietary sodium levels, the average body weight of broilers at 14 and 28 days of age increased significantly ( $P < 0.05$ ), and the average body weight of broilers in the low sodium group at 42 days of age was significantly lower than that in the medium and high sodium groups ( $P < 0.05$ ). The average daily gain of broilers in the low sodium group during the starter, grower, finisher, and overall periods was significantly lower than that in the medium and high sodium groups ( $P < 0.05$ ). The average daily feed intake of broilers during the starter phase increased significantly with increasing dietary sodium levels ( $P < 0.05$ ), while the average daily feed intake during the grower, finisher, and overall periods was significantly lower in the low sodium group compared to the medium and high sodium groups ( $P < 0.05$ ). The feed-to-gain ratio during the starter, grower, and overall periods was significantly higher in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). 2) The blood potassium concentration of broilers in the low sodium group at 28 days of age was significantly higher than that in the medium sodium group ( $P < 0.05$ ), while the blood sodium and chloride concentrations at 28 days of age in this group were significantly lower than those in the medium and high sodium groups ( $P < 0.05$ ). The blood sodium concentration of broilers in the low sodium group at 42 days of age was significantly lower than that in the medium

and high sodium groups ( $P < 0.05$ ). 3) Dietary sodium levels had no significant effect on hematological parameters of broilers at 28 days of age ( $P > 0.05$ ). The blood hemoglobin concentration in the high sodium group at 42 days of age was significantly higher than that in the low sodium group ( $P < 0.05$ ), and the blood hematocrit in the low sodium group was significantly lower than that in the medium and high sodium groups ( $P < 0.05$ ). 4) Dietary sodium levels had no significant effect on blood gas parameters of broilers ( $P > 0.05$ ). 5) The tibia weight and length of broilers in the low sodium group at 42 days of age were significantly lower than those in the medium and high sodium groups ( $P < 0.05$ ). In conclusion, a dietary sodium level of 0.075% reduced feed intake in broilers, which was detrimental to tibia development and growth; higher dietary sodium levels (0.245%) improved growth performance in broilers, with better effects observed during the starter phase.

## Full Text

### Effects of Dietary Sodium Level on Growth Performance, Hematological Parameters and Tibia Development of Broilers

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**Abstract:** This study was conducted to investigate the effects of dietary sodium level on growth performance, blood ion concentrations, blood routine, blood gas and tibia parameters of broilers. A total of 270 one-day-old healthy male Arbor Acres (AA) broilers were randomly assigned to 3 groups with 6 replicates per group and 15 broilers per replicate. Broilers in the 3 groups were fed diets with chlorine meeting requirements but sodium levels of 0.075% (low sodium group), 0.160% (medium sodium group) and 0.245% (high sodium group), respectively. The experiment lasted for 42 days, divided into early period (1-14 days of age), medium period (15-28 days of age) and late period (29-42 days of age). The average body weight at 14 and 28 days of age increased significantly with dietary sodium level ( $P < 0.05$ ). The average body weight at 42 days of age in the low sodium group was significantly lower than in the medium and high sodium groups ( $P < 0.05$ ). The average daily gain in the low sodium group was significantly lower than in the medium and high sodium groups during the early, medium, late and whole periods ( $P < 0.05$ ). Average daily feed intake during the early period increased significantly with dietary sodium level ( $P < 0.05$ ), while during the medium, late and whole periods, it was significantly lower in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). Feed/gain ratio during the early, medium and whole periods was significantly higher in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). 2) Blood potassium concentration at 28 days of age was significantly higher in

the low sodium group than in the medium sodium group ( $P < 0.05$ ), while blood sodium and chloride concentrations at 28 days of age were significantly lower in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). Blood sodium concentration at 42 days of age was significantly lower in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). 3) Dietary sodium level had no significant effect on blood routine parameters at 28 days of age ( $P > 0.05$ ). Blood hemoglobin concentration at 42 days of age was significantly higher in the high sodium group than in the low sodium group ( $P < 0.05$ ), while blood hematocrit was significantly lower in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). 4) Dietary sodium level had no significant effect on blood gas parameters ( $P > 0.05$ ). 5) Tibia weight and length at 42 days of age were significantly lower in the low sodium group than in the medium and high sodium groups ( $P < 0.05$ ). In conclusion, a dietary sodium level of 0.075% reduced feed intake and was detrimental to tibia development and growth, while a higher sodium level (0.245%) improved broiler growth performance, particularly during the early period.

**Keywords:** broilers; sodium; chlorine; dietary electrolyte balance; growth performance; hematological parameters; tibia development

## Introduction

Electrolytes are stable ions in metabolic processes, divided into cations and anions, with cations being alkaline and anions acidic. As animal nutrition research advances, the important role of dietary electrolytes in livestock growth and physiological-biochemical reactions has gained increasing attention from nutritionists. Sodium is a common cation, yet its optimal dietary level remains undetermined. The NY/T 33-2004 standard recommends a sodium requirement of 0.15%-0.20% for broilers. Some scholars suggest that 0.40% dietary sodium is ideal for broiler feed intake and body weight gain, while others report that 0.25% and 0.28% sodium are optimal during the starter phase and 0.15% sodium is best during the finisher phase. Recent studies by Koreleski et al. propose that low sodium (0.12%) yields better growth performance, and Jankowski et al. also recommend lower sodium levels in broiler diets (0.15% for 1-14 days, 0.11% for 15-35 days). Some researchers advocate considering both sodium and chloride through the dietary electrolyte balance (dEB) concept ( $dEB = Na + K - Cl$ ), suggesting that a dEB of 250 mEq/kg is suitable for broiler growth. Recent international reports recommend increasingly lower sodium levels for broilers, but domestic research on sodium requirements remains limited and inconsistent, focusing primarily on leg disorders. As an essential ion for poultry, sodium regulates growth metabolism, and appropriate dietary sodium levels are crucial for maximizing broiler growth potential. This study investigated the effects of dietary sodium level on broiler growth performance, blood ion concentrations, blood routine, blood gas and tibia parameters while meeting chlorine requirements, aiming to determine the appropriate sodium level for broiler diets and provide a scientific basis for broiler production.

## 1.1 Experimental Materials

Arbor Acres (AA) broilers were purchased from Langfang Kangda Poultry Breeding Co., Ltd. Sodium chloride (99.5%), sodium carbonate (99.8%), potassium chloride (99.5%), ammonium chloride (99.5%) and potassium carbonate (99.0%) were purchased from Xilong Chemical Co., Ltd.

## 1.2 Experimental Design and Diets

A total of 270 healthy 1-day-old AA male broilers with similar body weight were randomly assigned to low, medium and high sodium groups, with 6 replicates per group and 15 broilers per replicate. The low sodium diet met chlorine requirements through added sodium chloride, with a sodium level of 0.075%. The medium and high sodium diets were formulated by adding sodium carbonate to the low sodium diet to achieve sodium levels of 0.160% and 0.245%, respectively. Dietary chlorine levels were based on commonly recommended levels from previous studies and referenced against NRC (1994) broiler nutrient requirements and the “Feeding Standard of Chickens” (NY/T 33-2004). The medium sodium diet level was also based on common recommendations from previous studies and referenced against NRC (1994) and NY/T 33-2004. Basal diets were formulated according to NRC (1994) broiler nutrient requirements, NY/T 33-2004 and the “AA Broiler Management Manual.” Sodium and chlorine levels were adjusted by modifying the sodium and chlorine premix in the basal diet (containing NaCl, Na CO and corn). Dietary sodium was determined using GB/T 13885-2003 and chlorine using GB/T 6439-2007. Basal diet composition and nutrient levels are shown in Table 1 . The 42-day experiment was divided into three phases: early (1-14 days), medium (15-28 days) and late (29-42 days).

## 1.3 Management

Broilers had free access to feed and water with 24-hour lighting. Room temperature was maintained at 33°C for the first 3 days, then reduced by 2°C weekly until reaching 24°C, which was maintained thereafter. Management followed the “AA Broiler Management Manual” with routine vaccination and disinfection. The experimental house was well-ventilated, with temperature and humidity recorded daily and regular cleaning performed.

## 1.4 Measurements

**1.4.1 Growth Performance** At the start of the experiment (day 0) and on days 14, 28 and 42, broilers were weighed by replicate after fasting to calculate average body weight (ABW). Feed consumption was recorded by replicate during the early, medium, late and whole periods to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G). Mortality was recorded daily with body weight measured, and survival rate was calculated.

**1.4.2 Blood Routine Parameters** On days 28 and 42, one broiler per replicate was selected and 3 mL of anticoagulated blood was collected. Hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured using a Japanese XE-800i hematology analyzer (Sysmex Corporation, Japan).

**1.4.3 Blood Gas Parameters** At the end of the experiment, one broiler per replicate was selected. A 2 mL disposable sterile syringe was rinsed with sodium heparin anticoagulant, and 1.0-1.5 mL of arterial blood was collected via cardiac puncture through the left ventricle, immediately sealed with a rubber stopper. Arterial blood pH, oxygen partial pressure ( $pO_2$ ), carbon dioxide partial pressure ( $pCO_2$ ) and bicarbonate ion ( $HCO_3^-$ ) concentration were measured using a Roche COMAS B221 blood gas analyzer.

**1.4.4 Blood Ion Concentrations** On days 28 and 42, two broilers per replicate were selected and 5 mL of blood was collected from the wing vein into a procoagulant tube, centrifuged at 3,000 r/min for 10 minutes. The supernatant was transferred to 1.5 mL Eppendorf tubes and stored at  $-20^\circ\text{C}$ . Blood sodium, potassium and chloride concentrations were measured using the electrode method.

**1.4.5 Tibia Parameters** At the end of the experiment, one broiler per replicate was selected, slaughtered and the right tibia was extracted. Tibia length was measured with vernier calipers, then dried at  $65^\circ\text{C}$  for 48 hours, cooled to room temperature and weighed.

## 1.5 Statistical Analysis

Experimental data were analyzed using SPSS 16.0 software. Homogeneity of variance was tested, and data were analyzed using the ANOVA procedure. Multiple comparisons were performed using Turkey's method. Data are expressed as means and standard error of the mean (SEM), with  $P < 0.05$  considered statistically significant.

## 2.1 Effects of Dietary Sodium Level on Broiler Growth Performance

As shown in Table 2, the average body weight of broilers at 14 and 28 days of age increased significantly with dietary sodium level ( $P < 0.05$ ). The average body weight at 14, 28 and 42 days of age in the medium and high sodium groups was significantly higher than in the low sodium group ( $P < 0.05$ ). Additionally, the high sodium group had significantly higher average body weight at 14 and 28 days than the medium sodium group ( $P < 0.05$ ), while no significant difference

was observed between medium and high sodium groups at 42 days ( $P>0.05$ ). Average daily gain during the early, medium, late and whole periods showed no significant difference between medium and high sodium groups ( $P>0.05$ ), but both were significantly higher than the low sodium group ( $P<0.05$ ). Average daily feed intake during the early period increased significantly with dietary sodium level ( $P<0.05$ ). During the medium, late and whole periods, ADFI showed no significant difference between medium and high sodium groups ( $P>0.05$ ), but both were significantly higher than the low sodium group ( $P<0.05$ ). Feed/gain ratio during the early, medium and whole periods showed no significant difference between medium and high sodium groups ( $P>0.05$ ), but both were significantly lower than the low sodium group ( $P<0.05$ ). No significant differences were observed in survival rate during any period ( $P>0.05$ ).

## 2.2 Effects of Dietary Sodium Level on Blood Ion Concentrations

As shown in Table 3, at 28 days of age, blood sodium and chloride concentrations in the low sodium group were significantly lower than in the medium and high sodium groups ( $P<0.05$ ), with no significant difference between medium and high sodium groups ( $P>0.05$ ). Blood potassium concentration was significantly higher in the low sodium group than in the medium sodium group ( $P<0.05$ ), with no significant difference between these two groups and the high sodium group ( $P>0.05$ ). At 42 days of age, blood sodium concentration was significantly lower in the low sodium group than in the medium and high sodium groups ( $P<0.05$ ), with no significant difference between medium and high sodium groups ( $P>0.05$ ). Blood chloride concentration in the low sodium group was slightly lower than in the medium and high sodium groups, but the difference was not significant ( $P>0.05$ ). Blood potassium concentration showed a decreasing trend with increasing dietary sodium level, but differences were not significant ( $P>0.05$ ).

## 2.3 Effects of Dietary Sodium Level on Blood Routine Parameters

As shown in Table 4, at 28 days of age, blood MCHC showed an upward trend with increasing dietary sodium level, but differences were not significant ( $P>0.05$ ), and no significant differences were observed in other blood routine parameters ( $P>0.05$ ). At 42 days of age, blood hemoglobin concentration was significantly lower in the low sodium group than in the high sodium group ( $P<0.05$ ), and blood hematocrit was significantly lower in the low sodium group than in the medium and high sodium groups ( $P<0.05$ ). No significant differences were observed in blood hemoglobin concentration between the medium sodium group and the low or high sodium groups ( $P>0.05$ ), and no significant difference in hematocrit was observed between medium and high sodium groups ( $P>0.05$ ). No significant differences were found in MCV, MCH or MCHC among groups ( $P>0.05$ ).

## 2.4 Effects of Dietary Sodium Level on Blood Gas Parameters

As shown in Table 5 , dietary sodium level had no significant effect on blood gas parameters ( $P>0.05$ ), although arterial blood pH and pO<sub>2</sub> showed an increasing trend with increasing dietary sodium level.

## 2.5 Effects of Dietary Sodium Level on Tibia Development

As shown in Table 6 , tibia weight and length at 42 days of age were significantly lower in the low sodium group than in the medium and high sodium groups ( $P<0.05$ ), with tibia weight in the medium and high sodium groups approximately 40% higher than in the low sodium group.

## 3.1 Effects of Dietary Sodium Level on Broiler Growth Performance

This study demonstrated that increasing dietary sodium level significantly improved average body weight during the early and medium periods. Although no significant difference was observed between 0.245% and 0.160% sodium groups during the late period, the 0.245% sodium group showed an upward trend, indicating that higher dietary sodium levels improved broiler growth performance. Previous studies have shown positive correlations between dietary sodium level and body weight gain, feed intake and feed conversion ratio, consistent with our results. Research indicates that broilers require 0.20%-0.25% sodium during the starter phase, and even 0.26% sodium during the initial period. Our results also showed that the 0.245% sodium group achieved the best growth performance, with average body weight at 14, 28 and 42 days being 116 g, 507 g and 759 g higher than the 0.075% sodium group, and 31 g, 92 g and 81 g higher than the 0.160% sodium group, demonstrating the clear advantage of high sodium levels. Broilers in the 0.075% sodium group showed growth retardation, consistent with Kuchinski et al.'s report on 0.078% sodium, where body weight gain decreased with reduced dietary sodium. In this study, ADFI and ADG in the 0.075% sodium group were consistently lower than in the 0.245% and 0.160% sodium groups, and F/G during the early and medium periods was significantly higher, indicating that reduced feed intake was a major factor causing decreased growth performance in sodium-deficient broilers. Additionally, sodium deficiency may cause other abnormal physiological responses or reduce digestive efficiency, further exacerbating growth inhibition. Excessive sodium intake can cause sodium toxicity; Vieira et al. reported that sodium levels exceeding 0.48% caused high mortality in young broilers, and Kurtoğlu et al. noted that levels above 0.3% caused chloride deficiency in blood and kidneys, affecting health. Although 0.245% sodium in this study did not reach previously reported toxic levels, the high sodium group showed a trend toward reduced survival during 15-28 days, suggesting that high sodium may adversely affect survival in young broilers, though not at a statistically significant level.

### 3.2 Effects of Dietary Sodium Level on Blood Ion Concentrations

Sodium is primarily absorbed in the small intestine through active transport via three pathways: uncoupled absorption, coupled absorption and neutral NaCl absorption (the main form). Chloride absorption is closely related to sodium absorption, with neutral NaCl absorption being the primary mechanism for both ions. Consequently, increased dietary sodium significantly elevated blood sodium and chloride concentrations at 28 days and showed an upward trend for chloride at 42 days. According to the principle of electrical neutrality, potassium and sodium are both cations with an inverse relationship, so increased dietary sodium reduced blood potassium concentration at 28 days and showed a decreasing trend at 42 days. Wei et al. reported that adding sodium (as sodium sulfate) to basal diets had no significant effect on blood sodium concentration. In our study, when dietary sodium levels were 0.160%-0.245%, no significant differences were observed in blood sodium, potassium or chloride concentrations, consistent with previous research. However, when dietary sodium decreased to 0.075%, blood sodium concentration decreased significantly, likely due to sodium deficiency. Wei et al. also reported that blood potassium concentration decreased with increasing dietary sodium, consistent with our results. In this study, significant changes in blood sodium, potassium and chloride concentrations were observed in the low sodium group at 28 days, but at 42 days, only blood sodium concentration remained significantly different, while potassium and chloride showed no significant changes, possibly because broilers are more sensitive to short-term dietary sodium changes and gradually adapt through long-term regulation. Blood sodium and chloride concentrations in the high sodium group were intermediate between the low and medium sodium groups, indicating that the high sodium level (0.245%) did not cause blood ion metabolism disorders, and the birds remained in a relatively stable and healthy state.

### 3.3 Effects of Dietary Sodium Level on Blood Routine Parameters

Hemoglobin participates in oxygen transport, and hematocrit (Hct) indirectly reflects the physical state of red blood cells. Combined with Hb, Hct helps classify anemia morphologically and can be elevated during vomiting or diarrhea. No diarrhea was observed in this study, so the increased Hb and Hct in the 0.245% sodium group could not be attributed to diarrhea. Studies have shown that increasing dietary sodium significantly increases Hct and Hb, consistent with our results, suggesting that 0.245% sodium provides better blood oxygen transport capacity and is more conducive to broiler growth and development.

### 3.4 Effects of Dietary Sodium Level on Blood Gas Parameters

Acid-base balance is an important indicator of health status. Studies have found that blood pH above 7.6 or below 7.0 can cause mortality in laying hens. Wei et al. reported that dietary sodium level had no significant effect on blood gas parameters, consistent with our results. However, other research suggests that increasing dietary sodium elevates blood pH,  $\text{HCO}_3^-$  concentration, total  $\text{CO}_2$  ( $\text{TCO}_2$ ) and base excess (BE). The discrepancy with our results may be due to the smaller sodium level gradient in this study, which remained within the birds' self-regulating capacity. Our study observed increasing trends in arterial blood pH and  $\text{pO}_2$  with higher dietary sodium. Partial pressure of oxygen ( $\text{pO}_2$ ) is produced by physically dissolved  $\text{O}_2$  in blood, which then combines with Hb. As previously noted, higher dietary sodium significantly increased Hct and Hb, and the increasing trends in pH and  $\text{pO}_2$  support that 0.245% sodium provides better respiratory function and oxygen transport capacity, reducing the occurrence of respiratory acidosis.

### 3.5 Effects of Dietary Sodium Level on Tibia Development

Tibia development is crucial for broiler growth. Studies have shown that drinking water ion levels can affect leg disorders, and medical reports indicate that long-term abnormal sodium intake can cause osteoporosis. Wang et al. also found that electrolytes affect leg disorders by altering blood acid-base balance, oxygen transport efficiency and bone collagen metabolism. Our results showed that tibia weight and length were significantly lower in the 0.075% sodium group than in the 0.160% and 0.245% sodium groups. Although blood acid-base balance was not significantly altered in this study, reduced feed intake caused by sodium deficiency may be the direct cause of slowed growth and softened bone. The dEB concept remains a topic of interest, with the commonly recommended calculation method being  $\text{dEB} = \text{Na} + \text{K} - \text{Cl}$ . In broiler studies, many researchers recommend a dEB value around 250 mEq/kg (250, 274 and 260 mEq/kg) for optimal growth. Our results showed that the dEB of 272 mEq/kg group achieved better growth performance during the early period, while during the late period, no significant difference was observed between the dEB 236 mEq/kg and 272 mEq/kg groups. Gezen et al. reported that the dEB 256 mEq/kg group had higher tibia ash content than the dEB 170 mEq/kg group, consistent with our findings that broilers had higher tibia weight when dEB was 236-272 mEq/kg.

## Conclusion

1. A dietary sodium level of 0.075% reduced broiler feed intake and inhibited tibia development and growth performance.
2. The effects of dietary sodium level on blood ion concentrations were

temporary, and sodium levels of 0.075%-0.245% remained within the broilers' self-regulating range.

3. A higher dietary sodium level (0.245%) improved broiler growth performance by increasing feed intake, feed conversion ratio and blood oxygen transport capacity, with better effects during the early period.

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