Sodium and Chloride Requirements for 1-21 Day-Old Yellow-Feathered Broilers (Postprint)

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Abstract

This experiment aimed to investigate the sodium and chloride requirements and their mechanism of action in 1- to 21-day-old yellow-feathered broilers by studying the effects of different dietary sodium and chloride levels on growth performance, excreta moisture content, serum indices, and intestinal sodium-glucose cotransporter 1 (SGLT1) and sodium/hydrogen exchanger 2 (NHE2) mRNA expression levels. A total of 1,200 healthy, well-developed 1-day-old fast-growing Lingnan yellow-feathered male broilers were selected and randomly divided into 5 groups based on body weight, with 6 replicates per group and 40 birds per replicate. The control group was fed a corn-soybean meal basal diet (without additional sodium and chloride supplementation), while the remaining groups were designed with a sodium to chloride ratio of 1:1, using salt and sodium bicarbonate to replace a portion of zeolite powder, resulting in dietary sodium and chloride levels of 0.1%, 0.2%, 0.3%, and 0.4%, respectively. Birds had ad libitum access to feed and water throughout the experiment. The results showed that dietary sodium and chloride supplementation significantly improved average daily gain, average daily feed intake, average daily water intake, and excreta moisture content ($P<0.05$), and significantly reduced feed-to-gain ratio and mortality rate ($P<0.05$). Among these, average daily gain increased in a quadratic broken-line pattern with increasing dietary sodium and chloride levels ($R^2=0.966$, $P=0.009$), while excreta moisture content exhibited a quadratic curve change of initially increasing then decreasing with increasing dietary sodium and chloride levels ($R^2=0.954$, $P=0.046$). Dietary sodium and chloride supplementation significantly increased serum osmotic pressure ($P<0.05$) and decreased serum uric acid, glucose, total cholesterol, and triglyceride concentrations ($P<0.05$), but had no significant effect on serum cholecystokinin, ghrelin, and growth hormone concentrations ($P>0.05$). The 0.2% and 0.4% dietary sodium and chloride groups showed significantly lower duodenal NHE2 mRNA expression levels and Na^+-K^+-ATPase activity compared with the

control group (P<0.05). The 0.2% dietary sodium and chloride group exhibited significantly higher ileal SGLT1 mRNA expression level compared with the control and 0.4% groups (P>0.05). The results suggest that under the conditions of this experiment, based on growth performance as the evaluation index and according to ANOVA and multiple comparisons, the appropriate dietary sodium and chloride levels for 1- to 21-day-old fast-growing yellow-feathered broilers are both 0.2%; while estimation through nonlinear regression models indicated that the appropriate dietary sodium and chloride levels are both 0.14%.

Full Text

Sodium and Chlorine Requirements of Yellow-Feathered Broilers Aged from 1 to 21 Days

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Abstract

This experiment investigated the effects of dietary sodium and chlorine levels on growth performance, excreta moisture rate, serum parameters, and intestinal sodium-glucose cotransporter 1 (SGLT1) and sodium/hydrogen exchanger 2 (NHE2) mRNA expression in yellow-feathered broilers aged 1–21 days, and estimated their sodium and chlorine requirements and underlying mechanisms. A total of 1,200 one-day-old healthy, fast-growing Lingnan yellow-feathered male broilers were randomly allocated to 5 groups based on body weight, with 6 replicates per group and 40 birds per replicate. The control group received a corn-soybean meal basal diet without additional sodium or chlorine, while the other groups were fed diets with sodium and chlorine at a 1:1 ratio, achieved by isometrically replacing zeolite powder with salt and sodium bicarbonate to achieve dietary sodium and chlorine levels of 0.1%, 0.2%, 0.3%, and 0.4%, respectively. Birds had ad libitum access to feed and water throughout the experiment.

Results showed that dietary sodium and chlorine supplementation significantly increased average daily gain (ADG), average daily feed intake, daily water consumption, and excreta moisture rate (P<0.05), while significantly decreasing feed-to-gain ratio and mortality (P<0.05). ADG exhibited a quadratic broken-line increase with rising dietary sodium and chlorine levels (R^2=0.966, P=0.009),

whereas excreta moisture rate displayed a quadratic pattern of initial increase followed by decrease (R^2=0.954, P=0.046). Sodium and chlorine supplementation significantly elevated serum osmotic pressure (P<0.05) and reduced serum uric acid, glucose, total cholesterol, and triglyceride concentrations (P<0.05), but had no significant effects on serum cholecystokinin, ghrelin, or growth hormone levels (P>0.05). The 0.2% and 0.4% sodium and chlorine groups showed significantly lower duodenal NHE2 mRNA expression and Na^+-K^+-ATPase activity compared with the control group (P<0.05), while the 0.2% group exhibited significantly higher ileal SGLT1 mRNA expression than both the control and 0.4% groups (P<0.05).

These findings indicate that under the conditions of this experiment, based on growth performance and using variance analysis and multiple comparisons, the optimal dietary sodium and chlorine levels for 1–21 day-old fast-growing yellow-feathered broilers are both 0.2%. However, nonlinear regression model estimation suggested optimal levels of 0.14% for both minerals.

Keywords: yellow-feathered broilers; sodium; chlorine; nutrient requirement

Introduction

Sodium and chloride ions are the principal cation and anion in extracellular fluid, playing decisive roles in maintaining normal extracellular fluid osmotic pressure and volume, regulating acid-base balance, and facilitating nutrient digestion and absorption. With decreasing inclusion of animal-derived ingredients in poultry diets and the inherently low sodium and chlorine content in plant-based feedstuffs, salt is commonly used as an effective mineral supplement to meet animal requirements. Previous studies have demonstrated that increasing dietary sodium and chlorine levels significantly improves broiler average daily feed intake, feed conversion ratio, and body weight gain, while also altering excreta moisture rate. Elevated dietary sodium and chlorine significantly affect serum anion and cation concentrations, leading to increased serum osmotic pressure and enhanced SGLT1 expression, while reducing NHE2 expression and Na^+-K^+-ATPase activity, consequently influencing nutrient metabolism.

Current research on broiler sodium and chlorine requirements has primarily focused on growth performance and serum sodium, potassium, and chloride concentrations, with limited investigation into their effects on appetite regulation, nutrient metabolism, and expression of sodium-related transporters. No studies have reported sodium and chlorine requirements specifically for yellow-feathered broilers, and practical production often relies on White Leghorn standards (NRC) and empirical experience. To support efficient, standardized production of domestically developed yellow-feathered broilers, there is an urgent need to establish their specific sodium and chlorine requirements. This experiment aimed to investigate the effects of dietary sodium and chlorine levels on growth performance, excreta moisture rate, serum biochemical and endocrine pa-

rameters, intestinal SGLT1 and NHE2 mRNA expression, and Na^+-K^+-ATPase activity, thereby exploring the requirements and mechanisms for 1–21 day-old yellow-feathered broilers to provide a scientific basis for diet formulation.

Materials and Methods

1.1 Experimental Animals and Grouping A total of 1,200 one-day-old healthy, fast-growing Lingnan yellow-feathered male broilers were randomly divided into 5 groups based on body weight, with 6 replicates per group and 40 birds per replicate. Grouping ensured no significant differences in body weight among groups or replicates (P>0.05).

1.2 Experimental Design and Dietary Treatments A single-factor randomized design was employed. The control group (Group 1) received a corn-soybean meal basal diet, while other groups were formulated according to the *Feeding Standard of Chicken* (NY/T 33—2004) and NRC (1994) with a dietary sodium-to-chlorine ratio of 1:1. Sodium and chlorine levels of 0.1%, 0.2%, 0.3%, and 0.4% were achieved by isometrically replacing zeolite powder with salt and sodium bicarbonate. All diets were formulated to meet nutritional levels specified in the *Feeding Standard of Chicken* (NY/T 33—2004). Dietary composition and nutrient levels are presented in Table 1 .

1.3 Management The feeding trial was conducted in July 2015 at the Animal Nutrition Research Farm of Guangdong Academy of Agricultural Sciences. Birds were raised on floor pens with rice hull litter under uniform environmental conditions. Feed and water were provided ad libitum, with routine management and immunization protocols followed. Temperature and relative humidity were measured at 08:00, 14:30, and 20:00 daily, with averages calculated as daily mean values (temperature: $31.04\$\pm1.19°C; relativehumidity : 81.92\pm\$9.93\%$).

1.4 Sample Collection and Processing At 21 days of age, 2 healthy birds per replicate with body weight close to the group average were selected, weighed, and blood samples were collected via wing vein into non-anticoagulant tubes. After 30 minutes of clotting, serum was separated by centrifugation at 3,500 r/min for 10 minutes, aliquoted into 500 L EP tubes, and stored at -80°C for subsequent serum biochemical and endocrine analysis. Following slaughter, proventriculus and gizzard were excised for proventriculus pH determination. For groups 1, 3, and 5, intact ileum and duodenum were isolated, longitudinally opened, rinsed with cold phosphate-buffered saline, blotted dry, and approximately 1 cm segments were snap-frozen in liquid nitrogen and stored at -80°C for SGLT1 and NHE2 mRNA expression and Na^+-K^+-ATPase activity assays.

1.5 Measurement Indicators and Methods 1.5.1 Growth Performance

Mortality events were immediately recorded with dead bird weight and remaining feed quantified to eliminate effects on final results. Prior to each growth phase conclusion, feed was withdrawn at 20:00 while water remained available, and birds were weighed by replicate at 08:00 the following day. Feed consumption was recorded to calculate average daily feed intake, average daily gain, feed-to-gain ratio, and mortality rate.

1.5.2 Water Consumption

Daily water consumption was recorded at 08:30 by measuring remaining water from the previous day and fresh water added, with evaporation corrected using two water troughs of known volume. Daily water intake was calculated as water added minus remaining water.

1.5.3 Excreta Moisture Rate

During the final 5 days, 2 birds per replicate with body weight close to the average were placed in cages for excreta collection. Collected excreta were weighed, then oven-dried at 60°C to constant weight. Excreta moisture rate (%) = 100 × (pre-drying weight - post-drying weight) / pre-drying weight.

1.5.4 Proventriculus pH

A pH meter (DELTA320, Mettler-Toledo) probe was inserted into the proventriculus to measure pH at upper, middle, and lower regions, with the average value recorded. The pH meter was calibrated using standard buffer solutions of pH 4.01, 6.86, and 9.18 before use.

1.5.5 Serum Biochemical and Endocrine Parameters

Serum sodium, potassium, chloride, uric acid, glucose, total cholesterol, and triglyceride concentrations were determined using assay kits from Nanjing Jiancheng Bioengineering Institute with a microplate spectrophotometer (Bio-Rad 550, USA). Serum cholecystokinin, ghrelin, and growth hormone concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using kits from Beijing Fangcheng Biological Technology Co., Ltd. Serum osmotic pressure was calculated as: 2×sodium + 2×potassium + glucose + uric acid.

1.5.6 Duodenal Na^+-K^+-ATPase Activity

Approximately 0.1 g of duodenum was accurately weighed and homogenized in 9 volumes of physiological saline (1:9 w/v) under ice-water bath conditions. After centrifugation at 2,500 r/min for 10 minutes, the supernatant was collected and diluted 10-fold with physiological saline to 1% concentration. Total protein content was determined using a BCA protein assay kit (Thermo Fisher Scientific, USA), and Na^+-K^+-ATPase activity was measured using an ultra-micro Na^+-K^+-ATPase assay kit (Nanjing Jiancheng Bioengineering Institute). Na^+-K^+-ATPase activity was defined as the amount of enzyme that hydrolyzes ATP to produce 1 mol inorganic phosphorus per hour per milligram of tissue protein.

1.5.7 Ileal SGLT1 and Duodenal NHE2 mRNA Expression

Total RNA was extracted from tissue samples using TRIzol reagent and reverse-transcribed using the PrimeScript™ RT reagent Kit (RR047A, TaKaRa). Primers for SGLT1, NHE2, and the reference gene β-actin were designed using Prime Premier 5.0 software based on GenBank sequences and synthesized by Shanghai Sangon Biotech Co., Ltd. (Guangzhou). Primer sequences are listed in Table 2 . Real-time quantitative PCR was performed in 96-well plates using 2 L cDNA, 1 L forward primer, 1 L reverse primer, 10 L SYBR Green PCR Master Mix, and 6 L ddH_2O. Cycling conditions were: 95°C for 1 min; 40 cycles of 95°C for 15 s, gene-specific annealing temperature for 15 s, and 72°C for 40 s. Using β-actin as the internal reference and confirming similar amplification efficiencies, the $\Delta\Delta$Ct method was applied to quantify relative mRNA expression levels ($2^{-}\Delta\Delta$Ct).

1.6 Statistical Analysis Data were analyzed using the GLM procedure of SAS V8.0 software, with Duncan's multiple comparison test applied when significant differences were detected ($P<0.05$). When variance analysis and Duncan's test indicated significance, nonlinear regression analysis was performed according to Robbins et al. to estimate nutrient requirements, with the 95% value of the x-coordinate at the quadratic curve maximum representing the sodium and chlorine requirement for yellow-feathered broilers.

Results

2.1.1 Effects of Dietary Sodium and Chlorine Levels on Growth Performance As shown in Table 3 , dietary sodium and chlorine levels significantly affected average daily feed intake, average daily gain, feed-to-gain ratio, and mortality ($P<0.05$). The control group exhibited significantly lower average daily feed intake and average daily gain, and significantly higher feed-to-gain ratio compared with other groups ($P<0.05$). Maximum average daily feed intake and average daily gain were observed at 0.2% dietary sodium and chlorine, which were significantly higher than the control and 0.1% groups ($P<0.05$) but not significantly different from the 0.4% group ($P>0.05$). No significant differences in feed-to-gain ratio were detected among groups receiving $ $0.1% sodium and chlorine ($P>0.05$). Mortality in the control group was significantly higher than in all groups except the 0.4% group ($P<0.05$). These results indicate that under the conditions of this experiment, optimal growth performance in 1–21 day-old yellow-feathered broilers was achieved at dietary sodium and chlorine levels of 0.2%.

Based on Robbins et al., a quadratic broken-line regression model was established between dietary sodium/chlorine levels (x) and average daily gain (y) [Figure 1: see original paper]:

$y = -869.3(0.14 - x)^2 + 20.71$ ($x<0.14$); $y = 20.71$ (x\$ 0.14)($R^{2}=0.966, P=0.009)

This equation indicates that body weight gain reached a plateau at 0.14% dietary sodium and chlorine, representing the estimated requirement from nonlinear regression modeling—lower than the empirically determined optimum (0.2%).

2.1.2 Effects of Dietary Sodium and Chlorine Levels on Water Consumption and Excreta Moisture Rate Table 3 shows that average daily water consumption increased significantly with rising dietary sodium and chlorine levels ($P<0.05$), plateauing at 0.2–0.3% levels. Excreta moisture rate exhibited a quadratic relationship with dietary sodium and chlorine levels ($P=0.046$), peaking at 0.3% and being significantly higher in all treatment groups compared with the control ($P<0.05$).

A quadratic regression model between dietary sodium/chlorine levels (x) and excreta moisture rate (y) [Figure 2: see original paper] yielded:

$y = -553.29x^2 + 271.17x + 35.96$ ($R^2=0.954$, $P=0.046$)

The maximum point of this curve corresponded to 0.23% dietary sodium and chlorine (95% of x-coordinate value), slightly lower than the empirical optimum (0.3%).

2.2 Effects of Dietary Sodium and Chlorine Levels on Proventriculus pH As shown in Table 3, dietary sodium and chlorine levels did not significantly affect proventriculus pH ($P>0.05$).

2.3.1 Effects of Dietary Sodium and Chlorine Levels on Serum Sodium, Potassium, Chloride Content and Osmotic Pressure Table 4 demonstrates that serum sodium, potassium, and osmotic pressure exhibited quadratic broken-line responses to increasing dietary sodium and chlorine levels. The control group showed significantly lower serum sodium and osmotic pressure but significantly higher potassium compared with other groups ($P<0.05$). Within the 0.1–0.4% range, no significant differences in serum sodium, potassium, or osmotic pressure were observed among treatment groups ($P>0.05$). Serum chloride reached its highest value at 0.2% dietary sodium and chlorine, significantly higher than the control ($P<0.05$) but not different from other treatment groups ($P>0.05$). Based on these parameters, the empirically determined optimal dietary sodium and chlorine level was 0.1%, while nonlinear regression analysis estimated requirements of 0.13%, 0.18%, and 0.13% based on serum sodium, potassium, and osmotic pressure, respectively.

2.3.2 Effects of Dietary Sodium and Chlorine Levels on Serum Uric Acid, Glucose, Total Cholesterol and Triglyceride Content Serum uric acid, glucose, and triglyceride concentrations showed quadratic broken-line decreases with increasing dietary sodium and chlorine levels. At 0.1% dietary

sodium and chlorine, these parameters were significantly lower than in the control group ($P<0.05$) but not significantly different from other treatment groups ($P>0.05$). Total cholesterol was lowest at 0.1% dietary sodium and chlorine, significantly lower than the control and 0.3–0.4% groups ($P<0.05$) but not different from the 0.2% group ($P>0.05$). Based on these indices, the empirical optimum was 0.1%, while regression analysis estimated requirements of 0.13%, 0.21%, and 0.10% based on uric acid, glucose, and triglyceride, respectively.

2.3.3 Effects of Dietary Sodium and Chlorine Levels on Serum Cholecystokinin, Ghrelin and Growth Hormone Content Dietary sodium and chlorine levels did not significantly affect serum cholecystokinin, ghrelin, or growth hormone concentrations ($P>0.05$), although cholecystokinin tended to decrease and ghrelin tended to increase with rising dietary levels.

2.4 Effects of Dietary Sodium and Chlorine Levels on Intestinal SGLT1, NHE2 mRNA Expression and Na^+-K^+-ATPase Activity Table 5 shows that ileal SGLT1 mRNA expression increased initially then decreased with rising dietary sodium and chlorine levels, peaking at 0.2% and significantly higher than other groups ($P<0.05$). Duodenal NHE2 mRNA expression and Na^+-K^+-ATPase activity decreased significantly, with the control group showing higher values than other groups ($P<0.05$).

Discussion

3.1.1 Effects of Dietary Sodium and Chlorine Levels on Growth Performance The results demonstrate that increasing dietary sodium and chlorine levels significantly improved average daily feed intake and average daily gain in 1–21 day-old broilers, plateauing at 0.2% dietary sodium and chlorine, while feed-to-gain ratio decreased significantly, plateauing at 0.1%. These findings align with Murakami et al., who reported that average daily gain increased with dietary sodium and chlorine levels up to 0.25% and 0.20%, respectively. Oviedo-Rondón et al. similarly observed quadratic effects of dietary sodium and chlorine on 1–21 day-old broiler performance, with optimal levels of 0.28% and 0.25%, respectively. Vieira et al. reported significantly higher mortality at 0.48% dietary sodium. In this experiment, dietary sodium and chlorine supplementation at 0.1–0.3% reduced mortality by over 4 percentage points, confirming that appropriate sodium and chlorine levels significantly improve broiler growth performance.

Sodium and chloride ions are the primary extracellular cation and anion, crucial for maintaining normal extracellular fluid osmotic pressure and volume. These ions are converted to acids and bases in vivo; in excessively acidic or alkaline environments, metabolic processes prioritize regulation of excess acid or base, potentially impairing growth. Increasing dietary sodium and chlorine

elevated serum sodium, chloride, and osmotic pressure without significantly altering blood pH, though enhanced blood buffering capacity was evidenced by changes in bicarbonate, total carbon dioxide, and base excess. This suggests that dietary sodium and chlorine influence growth performance by modulating blood osmotic pressure and buffering capacity.

3.1.2 Effects of Dietary Sodium and Chlorine Levels on Water Consumption Increasing dietary sodium and chlorine levels significantly elevated water consumption in 1–21 day-old broilers, consistent with findings by Vieira et al., Maiorka et al., and Mushtaq et al. Elevated serum sodium, chloride, and osmotic pressure stimulate increased water intake to maintain homeostasis, with subsequent dilution of serum electrolytes and reduction in osmotic pressure.

3.1.3 Effects of Dietary Sodium and Chlorine Levels on Excreta Moisture Rate Conflicting results exist regarding dietary sodium and chlorine effects on excreta moisture. Murakami et al. reported a positive linear relationship with sodium but not chlorine, while Mushtaq et al. demonstrated combined effects of both ions. Koreleski et al. found positive correlations with potassium but not sodium. This experiment revealed a quadratic relationship between dietary sodium/chlorine and excreta moisture rate in 1–21 day-old broilers, peaking at 0.23% dietary sodium and chlorine. This pattern resembles serum chloride trends, though the precise relationship between serum chloride and water-electrolyte metabolism requires further investigation.

3.2 Effects of Dietary Sodium and Chlorine Levels on Proventriculus pH Proventriculus pH is primarily determined by gastric acid, formed from hydrogen ions secreted via gastric proton pumps and chloride ions transported across the apical membrane, and critically influences gastric enzyme activity. No previous reports have addressed dietary sodium and chlorine effects on proventriculus pH; this experiment found no significant impact.

3.3.1 Effects of Dietary Sodium and Chlorine Levels on Serum Biochemical Parameters Sodium, potassium, and chloride are the primary ions maintaining blood osmotic pressure and strong ion difference. This experiment demonstrated that increasing dietary sodium and chlorine significantly elevated serum sodium and chloride while reducing potassium, with these parameters plateauing at 0.1% dietary sodium and chlorine. Mushtaq et al. reported linear increases in serum sodium with dietary chlorine, and Bao et al. observed elevated serum sodium in ducks fed high-chlorine diets. These findings support the principle that under normal conditions, extracellular sodium, bicarbonate, and chloride maintain a relatively constant relationship (sodium bicarbonate + chloride + anion gap). Elevated serum chloride induces corresponding increases in serum sodium to maintain electroneutrality. In this experiment, dietary sodium was provided by salt and sodium bicarbonate; as dietary sodium and

chlorine increased from 0 to 0.42% bicarbonate with equal sodium and chloride, electroneutrality principles dictated that high bicarbonate would displace part of chloride, evidenced by declining serum chloride beyond 0.22% dietary bicarbonate. Regarding potassium, with fixed dietary potassium levels, sodium became the dominant cation as dietary sodium increased, leading to decreased serum potassium according to electroneutrality principles.

Bao and Wang reported higher serum uric acid in ducks fed low- or high-chlorine diets. This experiment similarly found decreasing serum uric acid with increasing dietary sodium and chlorine, plateauing at 0.1%. High serum uric acid at low dietary sodium and chlorine indicates increased endogenous nitrogen excretion and impaired deposition, disrupting protein metabolism and amino acid balance. Xu et al. reported that insulin promotes amino acid and glucose uptake via membrane transport systems when blood glucose is abundant, enhancing protein and glycogen synthesis. As glucose serves as a crucial substrate for protein synthesis, blood glucose concentration reflects carbohydrate metabolic status—high levels indicate metabolic disturbance, while stable levels suggest balance. This experiment showed significant reductions in serum glucose and triglycerides with increasing dietary sodium and chlorine, with total cholesterol decreasing initially then increasing, all plateauing at 0.1% dietary sodium and chlorine. These results suggest that low dietary sodium and chlorine may affect protein, carbohydrate, and lipid metabolism by modulating serum uric acid, glucose, triglycerides, and total cholesterol, though this regulatory effect diminishes beyond 0.1%, indicating the body' s capacity to maintain metabolic stability.

In summary, increasing dietary sodium and chlorine induced reciprocal adjustments among serum sodium, potassium, chloride, and bicarbonate to maintain electroneutrality and osmotic stability, with serum osmotic pressure stabilizing beyond 0.1% dietary sodium and chlorine. Nutrient metabolism was also affected, with glucose metabolism being most sensitive. The similar trends among serum osmotic pressure, growth performance, and glucose metabolism suggest that dietary sodium and chlorine may influence nutrient metabolism and growth performance through modulation of serum osmotic pressure.

3.3.2 Effects of Dietary Sodium and Chlorine Levels on Serum Endocrine Parameters Salt functions as a flavoring agent with distinct salty taste that intensifies with concentration, playing an important role in appetite regulation. Cholecystokinin, a gastrointestinal peptide hormone, inhibits feeding behavior and affects hormone secretion, with both active and passive immunization against cholecystokinin increasing feed intake and weight gain. Ghrelin, a gastric peptide and endogenous ligand for growth hormone secretagogue receptor 1A, regulates appetite, feeding, and body composition, and can enhance gastric motility and emptying by modulating gastric acid and digestive enzyme secretion, thereby increasing body weight. This experiment showed that while dietary sodium and chlorine significantly increased average daily feed intake, serum cholecystokinin and ghrelin concentrations were not significantly

affected. Thus, under these experimental conditions, increased dietary sodium and chlorine did not regulate feed intake through these hormones, possibly acting via other appetite-regulating factors, though the exact mechanism requires further investigation. Dietary sodium and chlorine also did not significantly affect serum growth hormone, indicating no influence on growth performance through growth hormone modulation.

3.4 Effects of Dietary Sodium and Chlorine Levels on Intestinal SGLT1, NHE2 mRNA Expression and Na^+-K^+-ATPase Activity SGLT1 is the primary determinant of glucose absorption in the small intestine. Low dietary sodium did not alter SGLT1 mRNA expression, though Garriga et al. found reduced SGLT1 expression in ileum and rectum but not jejunum of chickens fed low-sodium diets, with expression restored upon sodium resalination. These transcriptional effects are termed post-transcriptional regulation, potentially mediated by aldosterone, which may be key to dietary sodium effects on SGLT1. In contrast, this experiment found that ileal SGLT1 mRNA expression increased then decreased with rising dietary sodium and chlorine, peaking at 0.2%. This discrepancy may relate to bird age, as previous studies used older birds, suggesting that transcriptional regulation capacity may strengthen with age while post-transcriptional regulation capacity does not.

NHE proteins mediate electroneutral exchange of extracellular sodium and intracellular hydrogen, with 9 isoforms identified. Wang et al. reported highest NHE2 mRNA expression in broiler duodenum, while Donowitz et al. found significantly higher colonic NHE2 mRNA expression in chickens fed low-sodium diets. Similarly, this experiment demonstrated significantly decreased duodenal NHE2 mRNA expression with increasing dietary sodium and chlorine.

Na^+-K^+-ATPase catalyzes ATP hydrolysis to ADP and phosphate, providing energy for active transport of sodium, chloride, hydrogen, and glucose. Na^+-K^+-ATPase activity decreases with increasing dietary sodium, consistent with this experiment' s findings of reduced duodenal Na^+-K^+-ATPase activity. These responses represent physiological adaptations to dietary sodium and chlorine levels, likely regulated by hormones such as aldosterone, growth hormone, cortisol, and insulin-like growth factors. The divergent trend between SGLT1 mRNA expression and growth performance suggests SGLT1 participates in but is not the primary mechanism regulating growth performance. Decreased NHE2 expression and Na^+-K^+-ATPase activity may inhibit sodium absorption and hydrogen secretion, indicating their potential role in maintaining osmotic and acid-base balance.

3.5 Discussion on Broiler Sodium and Chlorine Requirements Livestock nutrient requirements are typically evaluated using dose-response methods, measuring sensitive indicators such as growth performance, blood parameters, and gene expression across graded nutrient levels, followed by comprehen-

sive judgment. However, requirements vary depending on the indicator used. Oviedo-Rondón et al. recommended optimal dietary sodium and chlorine levels of 0.28% and 0.23% for 1–21 day-old broilers based on growth performance, while blood gas analysis suggested optimal chlorine of 0.19%. This experiment combined traditional methods with nonlinear regression modeling, yielding different optimal levels based on growth performance, serum osmotic pressure, and glucose concentration.

Synthesizing these discussions, dietary sodium and chlorine influence nutrient metabolism (with glucose being most sensitive) and growth performance by modulating blood osmotic pressure and acid-base balance. Therefore, growth performance, serum osmotic pressure, and glucose concentration were selected as criteria to determine sodium and chlorine requirements (Table 6). The requirements estimated from average daily gain and serum glucose were consistent with NRC (1994) recommendations for broilers but significantly higher than the Chinese Feeding Standard for Yellow-Feathered Broilers (2004). Requirements estimated from average daily gain and serum osmotic pressure were substantially lower than NRC (1994), Murakami et al., and Oviedo-Rondón et al. recommendations. These discrepancies suggest that breed, management practices, and evaluation criteria influence sodium and chlorine requirements, necessitating appropriate standard selection for practical diet formulation.

Table 6. Requirements of sodium and chlorine for yellow-feathered broilers from 1 to 21 days of age by different evaluation criteria

Evaluation indices	Factorial estimate	Estimate from regression analysis
Growth performance (ADG)	0.2%	0.14%
Serum osmotic pressure	0.1%	0.13%
Serum GLU content	0.1%	0.14%

Conclusions

Under the conditions of this experiment:

1. Dietary sodium and chlorine supplementation significantly increased average daily gain, feed intake, water consumption, and excreta moisture rate while reducing mortality, with average daily gain showing a quadratic broken-line response to increasing dietary sodium and chlorine levels.
2. Sodium and chlorine supplementation significantly elevated serum osmotic pressure, reduced serum uric acid, glucose, total cholesterol, and triglyc-

eride concentrations, decreased ileal SGLT1 mRNA expression, and suppressed duodenal NHE2 mRNA expression and Na^+-K^+-ATPase activity.

3. Based on growth performance and nonlinear regression modeling, the optimal dietary sodium and chlorine levels for 1–21 day-old fast-growing yellow-feathered broilers were estimated to be 0.14%.

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Note: Figure translations are in progress. See original paper for figures.