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Research Article

Effects of Varying Free Chlorine Residuals in Poultry Drinking Water on Early Performance and Amino Acids Digestibility of Broiler Chickens

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Abstract

Background and Objective: Chlorine is a commonly utilized poultry drinking water sanitizer; however, little is known about the impact of varying usage levels on bird performance. This study was conducted to understand the performance impacts of various levels of field-practiced chlorine-based product (8.25% sodium hypochlorite) in poultry drinking water. **Materials and Methods:** A total of 100 Cobb 500 byproduct males were fed a standard experimental diet from day 0-11. Five treatment groups (20 birds/treatment) were created based on chlorine-product dosage levels in drinking water that mimicked field practice and above-the-field practice levels: No product treatment (T1), treatments with product concentrations of 0.5, 1, 2 and 4 mL per 25 mL of drinking water (T2-T5) and stock mixed at 1:100 with drinking water. T4 and T5 represented mixing rates above field practice levels for poultry drinking water supplies that produced free chlorine residuals in water up to 21 ppm immediately after mixing. The average free chlorine residual retained in ppm until 24 hrs were 0.25, 0.42, 0.67 and 0.83 ($p>0.05$). Data were analyzed using one-way ANOVA. **Results:** No significant difference ($p>0.05$) was observed in body weight gain among the treatment groups. Water consumption was not different among treatment groups ($p>0.05$), however, improved FCR (0.94) was noted in chickens fed with the T3 group ($p<0.05$). **Conclusion:** There were no differences ($p>0.05$) in the apparent ileal digestibility of amino acids across treatments, ranging from 0.44-0.93. None of the other treatment groups for various mixing rates of chlorine produced adverse effects on bird performance except for T5.

Key words: Chlorine levels, growth performance, microbial levels, sanitation, water supplies

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The poultry industry is experiencing remarkable growth globally as consumer demand rises for poultry products (meat and eggs) as a source of protein[1]. Within this dynamic sector, the health and well-being of broiler chickens at the farm level is of paramount importance, as it is directly linked to consumer health[2]. One critical factor that significantly influences broiler health and overall performance is the level of microbial contamination in drinking water[3]. Among the different chemical-based poultry sanitizers, the use of chlorine to sanitize poultry drinking water supplies is gaining popularity, which has been attributed to its minimal impact on the nutritional and aesthetic quality of the product, coupled with its readily available and user-friendly application[4]. Furthermore, the efficacy of chlorine is well-known for disinfection, as it effectively inactivates bacteria, viruses and protozoa, thereby reducing the risk of cross-contamination. Chlorine disinfection is influenced by various factors, including the water's pH, turbidity, the chlorine concentration, contact surface and the contact time and presence of organic materials. Upon introduction to water, chlorine undergoes rapid hydrolysis, resulting in the formation of hypochlorous acid (HOCl) and hydrochloric acid. HOCl, being a weak acid, undergoes partial dissociation into hypochlorite ion (OCl-)[5,6]. Among chlorine-based disinfectants used in the poultry industry, sodium hypochlorite (NaOCl) stands out as a primary and extensively utilized disinfectant due to its ease of application, strong sterilization power and cost-effectiveness[7]. Chlorine can become toxic when utilized beyond recommended levels. For effective water sanitization in drinkers at the end of the poultry house, it has been found to be advisable to use free chlorine in water within the range of 2-3 ppm[8]. In this study, the use of sodium hypochlorite for sanitizing poultry water supplies and equipment, adhering to the commonly recommended levels of 2-5 ppm chloride, was anticipated to pose no adverse effects on the performance of broiler chicks or laying hens. However, previous research suggests that 50 ppm of chlorine in water can reduce water consumption[9]. Maharjan *et al.*[10] have emphasized the importance of line cleaning between flocks and maintaining daily water free chlorine levels between 0.5 and 1 ppm at the beginning of waterlines, with an oxidation-reduction potential (ORP) exceeding 600 mV to inhibit microbial growth (<1000 CFU/mL) during the grow-out period.

In this study, we chose a commonly utilized chlorine-based product (8.25% sodium hypochlorite) and tested out its usage levels in water that mimicked the field practice and a few folds (up to 4X) above field practice usage levels. The goal was to set an analogy to determine whether bird performance would be affected if growers used 2X or 8X higher product dosages than field practice level, either mistakenly or deliberately, due to chronic exposure of higher free chlorine levels. The test assessed the early grow-out period in broiler chickens.

MATERIALS AND METHODS

The study was conducted at the Poultry Research Unit, College of Agriculture, Tennessee State University, Nashville, Tennessee. All experimental procedures were conducted in accordance with the guidelines set by the Local Experimental Animal Care Committee (ACUP-2402) and received approval from the College's ethics committee.

Preparation of stock solution and creation of drinking water treatments: The municipal water source was utilized in the study. To ensure there was no free chlorine residual in the tap water (as detected by Water Works™ free chlorine test strips), it was first allowed to stand overnight before undergoing treatment with various doses of chlorine-based product (8.25% sodium hypochlorite (NaOCl) to create stock solutions. The following drinking water treatments were created: No product treatment (control, T1), treatments with product concentrations of 0.5, 1, 2 and 4 mL per 25 mL of municipal drinking water source (T2-T5, respectively) and treatments in which each stock solution was mixed at 1-100 mL municipal water as the end drinking water preparation. The mineral profile for the municipal source (Table 1) was monitored, as was the free chlorine level in the treatment water (in triplicates) at various time intervals: 0, 1, 2, 4, 8 and 24 hrs post mixing the stock solution. Free chlorine test strips (Water Works™) were utilized.

Table 1: Mineral profiles of different sources of water

	Minerals ppm (mg/L)											
	Ca	Mg	Fe	Mn	Cl	Na	SO_4^{2-}	S	Pb	Cu	Zn	pH
Tested concentrations	32.3	5.4	N.D.	N.D.	14.2	9.7	32.2	10.8	<0.33	<0.04	0.06	6.83
*Unacceptable levels	121#	125	0.3	0.05	150	150	200	-	0.05	0.6	1.5	8

N.D: Not detected, *Values exceeding acceptable limits and deemed unacceptable for the respective minerals. The values presented in the last row are sourced from Koelkebeck[11] and #No upper limit for bird safety and Limit set at 110 mg/L for equipment functionality

Birds, design and experimental diets: A total of 100 Cobb-500 byproduct males (20 birds/treatment) were fed a common starter diet as recommended by primary breeder nutrition guidelines (Table 2). The experiment was conducted over the early phase of the grow-out period, (day 0-10). Birds were reared as per the basic husbandry guidelines for the breed. Treatment water (T1-T5) was given throughout the experimental period. Biosecurity measures were strictly followed while conducting daily bird care.

Performance parameters and water consumption: Individual body weight and feed intake were recorded at the time of placement (day 0), on day 7 and on day 10. These data were utilized to measure feed conversion for treatment groups. Daily water consumption was measured for each treatment group by calculating the difference between water output and water input, then per-bird water consumption was recorded.

Amino acid digestibility: On day 10, three birds per treatment group were sacrificed. Ileal digesta were collected (lower half to Meckel's diverticulum) and snap frozen using liquid nitrogen, then stored in a freezer at -64°C and later subjected to freeze-drying for nutrient analysis. Amino acid and titanium in digesta and feed samples were assessed. Apparent ileal digestibility of amino acids was calculated using the formula:

$$AID = 1-[(Ci/Co)\times(Xo/Xi)]$$

where, Ci represents the concentration of TiO_2 in the diet, Co denotes the concentration in digesta, Xo signifies the amino acid content in digesta and Xi represents the amino acid content in the diet[10].

Data analysis: For treatment groups, the variables assessed were body weight gain, feed conversion, amino acid digestibility and water consumption. The data analysis was conducted using a one-way ANOVA to assess the significance between the means and Duncan's multiple ranges test was utilized[12]. Statistically significant differences were set at a p-value less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Free chlorine levels in drinking water: No chlorine was detected on any occasion in T1, where no chlorine was added (Table 3). Conversely, the highest free chlorine reading was recorded in T5 at 0 hrs, reaching 21.67 ppm, which subsequently decreased to 0.83 ppm after 24 hrs. Notably, each treatment with a higher concentration relative to the

Table 2: Ingredient and nutrient composition of experimental diets

Ingredients		Inclusion (%)
Poultry premix 3%		0.1
Dicalcium phosphate		3.8
Corn		55.0
Lysine HCl		0.8
DL methionine		0.8
NaCl		0.4
Soybean meal 48		36.1
Sodium selenite		0.0
Animal fat		3.0
Titanium dioxide[1]		0.5
Nutrient composition	**Calculated value (%)**	**Analyzed value (%)**
Metabolizable (MJ/kg)		13.21
Crude Protein		24.00
Lysine		1.97
Methionine	12.4	0.98
Calcium	21.0	
Sodium	1.6	
Available P	0.5	
Chloride	0.9	
Potassium	0.2	
Taurine	0.5	0.18
Hydroxyproline	0.2	0.03
Aspartic acid	0.6	2.42
Threonine		0.87
Serine		0.96
Glutamic acid		4.27
Proline		1.27
Glycine		0.98
Alanine		1.11
Cysteine		0.36
Valine		1.18
Isoleucine		1.07
Leucine		1.90
Tyrosine		0.77
Phenylalanine		1.16
Hydroxylysine		0.02
Ornithine		0.02
Histidine		0.62
Arginine		1.54
Tryptophan		0.28

The poultry premix used in the experimental diets was manufactured by Akey Nutritional Products, HCL, hydrochloric acid; NaCl, sodium chloride, P: Phosphorous and [1]Titanium dioxide was incorporated into diet at the rate of 1:200

Table 3: Free chlorine levels (ppm) in drinking water detected over different occasions

Hours	T1	T2	T3	T4	T5
0	0.25^{d}	1.67^{cd}	5.33^{bc}	8.33^{b}	21.67^{a}
1	0.08^{d}	1.67^{cd}	2.67^{c}	5.67^{b}	8.33^{a}
2	0^{d}	1.00^{cd}	1.67^{c}	3.67^{b}	6.33^{a}
4	0^{d}	0.5^{cd}	1.00^{bc}	1.67^{b}	2.67^{a}
8	0^{c}	0.41^{bc}	0.83^{ab}	0.83^{ab}	1.00^{a}
24	0^{c}	0.25^{bc}	0.42^{abc}	0.67^{ab}	0.83^{a}

T1 represents control water, 0 free chlorine residual, T2-T5 represents 0.5, 1, 2, 4 mL 8.25% sodium hypochlorite per 25 mL of drinking water to prepare stock solutions and then stock mixed at 1 to 100 mL of drinking water, Analysis was conducted in triplicates and Different letters in superscripts represent significantly different means between different treatment groups within line in each column

Table 4: Effect of NaOCl dose on broiler body weight gain, feed intake and feed conversion ratio

Parameters	T1	T2	T3	T4	T5	p-value
BWG g, day 0-10	190.23	192.11	216.95	174.00	172.40	p>0.05
FI per bird, day 0-10	326.30	331.72	340.49	336.53	334.70	p<0.05
FCR in day 10	1.72	1.73	1.57	1.93	1.94	p<0.05

T1 represents control water, 0 free chlorine residual. 'T2-T5' represents 0.5, 1, 2, 4 mL 8.25% sodium hypochlorite per 25 ml of drinking water to prepare stock solutions and then stock mixed at 1 to 100 mL of drinking water

others exhibited correspondingly higher free chlorine readings, aligning with their respective concentrations. It's possible that the concentration of free chlorine decreases over time when chlorinated water is stored[13]. Free chlorine concentration decreases over time due to various factors, including consumption and reactions with constituents in the water, such as deposits, corrosion by-products, microorganisms, organic impurities and metallic compounds like iron and manganese[14]. Common chlorination practices require adequate levels of free chlorine and sufficient contact time to meet regulatory standards for removing pathogenic microorganisms from drinking water while preventing their regrowth during the storage and distribution processes[15].

Growth performance and water consumption: The effect of free chlorine residual concentration on the growth performance parameters for treatment groups is presented in Table 4. Higher body weight gain was detected in T3, with a value of 204.95 g. The highest feed intake was observed in birds supplied with T3. The improved FCR (1.66) was observed in the T3 birds. Overall, this shows that the water treated with T3 exhibited superior flock performance. Hulan and Proudfoot[16] conducted similar studies in Shaver broiler chicks and observed that positive performance of birds was correlated with lower concentrations of free chlorine, even though it was much higher (~37.5 ppm) than the free chlorine levels tested in this study. The negative effects reported in the same study when adding available chlorine at a higher dose (1200 ppm) were higher mortality, reduced feed efficiency, lowered water consumption and diminished weight of heart, liver, kidney and testes. Similarly, in chick experiments, the inclusion of 100 ppm chloride in poultry drinking water was associated with reduced water intake, while the presence of 300 ppm chloride resulted in a decreased body weight[9]. The reduced water consumption and water/feed consumption ratio of broiler chicks given commercial water may be attributed to higher chloride levels in the water[17]. The supplementation of drinking water with sodium hypochlorite at various dose levels, starting from 25 mg chlorine/L up to 400 mg chlorine/L, did not result in any observable clinical signs of toxicity in Japanese quails over a six-week period. However, when the dose level was increased to 100 mg chlorine/L and subsequently increased fourfold every week up to 6400 mg chlorine/L by the 10th week, researchers observed prominent clinical signs of depression and anorexia, accompanied by decreased feed intake, body weight and alterations in hematological parameters[18]. This indicates that higher concentrations of sodium hypochlorite in drinking water can adversely affect the health and performance of poultry.

A daily trend of increasing water consumption was observed from day 0-9 (Fig. 1). As chickens mature, they may experience higher water-loss rates through respiration, excretion and other physiological processes. To maintain proper hydration and physiological balance, older chickens need to recover the lost water through increased consumption[19]. However, there was a notable spike in water consumption from day 8 to 9 compared to the preceding days. Total water consumption was highest in the T3 group, with an average of 567.50 mL per bird during the experimental period (Fig. 2). It is important to note that the water needs of broiler birds are influenced by factors such as environmental temperature, relative humidity, diet composition, growth rate and the efficiency of kidney water resorption[20].

Amino acid digestibility: Amino acid digestibility in feed due to treatment effects was not different for individual amino acids (p>0.05) and ranged from 0.44-0.93 (Table 5). The essential amino acids included in the analysis were arginine, glycine, histidine, isoleucine, leucine, threonine, lysine and phenylalanine; non-essential amino acids were aspartic acid, serine, glutamic acid, proline, alanine, valine and tyrosine. It's interesting to note that for most amino acids, T3 resulted in the highest digestibility values numerically compared to other treatments. However, hydroxylysine exhibited the highest digestibility value in T2, contrary to the general trend observed for other amino acids. This deviation highlights the complexity of amino acid metabolism and the potential for specific amino acids to respond differently to environmental factors such as water treatment[21]. A previous study conducted by Yadav and Jha[22] highlighted the antimicrobial properties of chlorine, as evidenced by the significantly reduced bacterial load in broiler chicks that consumed chlorinated drinking water compared to untreated water, which could have gut

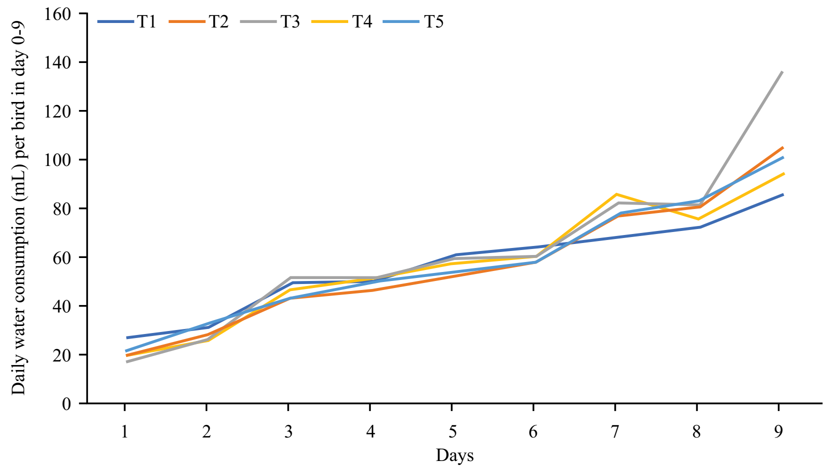

Fig. 1: Daily water consumption of broiler chickens throughout the grow-out period (day 9) at different treatment sources

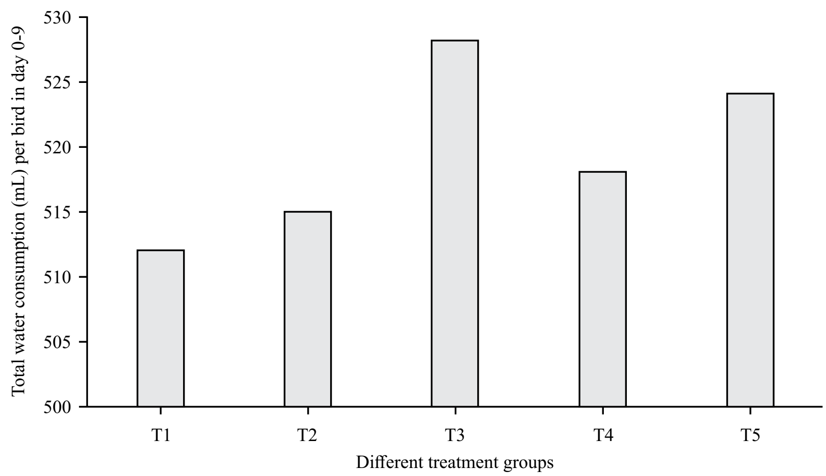

Fig. 2: Total water consumption per chick throughout the grow-out period (day 9) at different treatment sources

Table 5: Standardized ileal digestible amino acids ("as is" basis) of experimental diets at day 10

Amino acid	T1	T2	T3	T4	T5
Taurine	0.45	0.57	0.55	0.54	0.44
Aspartic acid	0.76	0.85	0.86	0.84	0.81
Threonine	0.66	0.78	0.81	0.78	0.74
Serine	0.74	0.83	0.85	0.83	0.81
Glutamic acid	0.82	0.89	0.91	0.89	0.88
Proline	0.74	0.82	0.84	0.82	0.79
Glycine	0.70	0.81	0.83	0.81	0.78
Alanine	0.74	0.84	0.86	0.84	0.82
Valine	0.74	0.84	0.85	0.84	0.81
Isoleucine	0.76	0.85	0.87	0.85	0.83
Leucine	0.75	0.84	0.86	0.84	0.82
Tyrosine	0.77	0.86	0.88	0.86	0.84
Phenylalanine	0.77	0.86	0.87	0.86	0.84
Hydroxylysine	0.62	0.69	0.68	0.67	0.61
Ornithine	0.81	0.69	0.84	0.67	0.61
Lysine	0.82	0.90	0.92	0.91	0.90
Histidine	0.78	0.86	0.88	0.87	0.84
Arginine	0.83	0.91	0.93	0.91	0.90

T1: Treatment 1, T2: Treatment 2, T3: Treatment 3, T4: Treatment 4, T5: Treatment 5, p-values for amino acid digestibility among treatments were not different (p>0.05), *Non-proteinogenic amino acids and Results are expressed on an "as-received" basis unless otherwise indicated health benefits. By maintaining a well-balanced gut microbiota, modifying cecal microbiota composition and promoting the production of beneficial metabolic products, chlorine could contribute to enhanced nutrient digestion, absorption, metabolism and overall health[23].

CONCLUSION

The use of a chlorine-based product producing as high as 21.67 ppm in poultry drinking water supplies did not affect amino acid digestibility in broilers. The application of a chlorine-based product in poultry drinking water supplies producing free chlorine residual as high as 5.3 ppm immediately after mixing could promise better early performance. Moreover, going beyond 5 ppm does not show improvement in bird performance, with no negative performance effects until 21.67.

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