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Effect of Giving Sea Salt Through Drinking Water to Broilers on the Total Aerobic Count of Bacteria, Coliforms, and *Escherichia coli* in Broiler Feces

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Abstract. The gastrointestinal microbiota plays an essential role in maintaining intestinal health, immune function, and productivity in broiler chickens. The growing concern about antimicrobial resistance arising from the prolonged use of antibiotic growth promoters has spurred exploration of natural alternatives to modulate gut microbial populations. Sea salt contains various minerals, including sodium, magnesium, potassium, and calcium, which may influence intestinal microbial ecology. This study aimed to evaluate the effect of sea salt supplementation through drinking water on the Total Aerobic Bacterial Count (TABC), Coliforms, and *Escherichia coli* populations in broiler feces. A total of 72 CP 707 broiler chickens were assigned to four treatment groups: control without sea salt supplementation (S0), 1 g/L sea salt (S1), 2 g/L sea salt (S2), and 4 g/L sea salt (S3). Fecal samples were collected by cloacal swabbing at 0, 15, and 30 days of age. Bacterial enumeration was performed using Nutrient Agar and Eosin Methylene Blue Agar. Data were analyzed using multivariate analysis of variance (MANOVA) followed by Duncan's Multiple Range Test. The results showed that sea salt supplementation had no significant effect on TABC, Coliform, or *E. coli* counts ($P>0.05$). In contrast, broiler age significantly affected all bacterial parameters evaluated ($P<0.05$). Although lower bacterial counts were numerically observed in the 1 g/L and 2 g/L treatment groups compared with the control, these differences were not statistically significant. In conclusion, sea salt supplementation through drinking water at concentrations up to 4 g/L did not significantly reduce fecal bacterial populations in broiler chickens. Age-related microbial succession had a greater influence on bacterial dynamics than sea salt supplementation.

Keywords: Broiler; Coliform; *Escherichia coli*; Sea salt; TABC

I. INTRODUCTION

Broiler chickens are among the most important livestock commodities, supplying affordable, high-quality animal protein to the growing human population. The poultry industry has experienced substantial expansion over recent decades due to rising demand for chicken meat, shorter production cycles, and higher feed conversion efficiency. However, the intensification of broiler production systems has also increased the susceptibility of birds to various health challenges, particularly those associated with gastrointestinal disorders and bacterial infections [1, 2].

The gastrointestinal tract of broiler chickens harbors a complex and dynamic microbial ecosystem that contributes to digestion, nutrient absorption, immune system development, pathogen exclusion, and maintenance of intestinal integrity [3, 4]. A balanced gut microbiota is therefore essential for maintaining optimal growth performance and health status. Conversely, disturbances in microbial composition may lead to dysbiosis, impaired nutrient utilization, increased susceptibility to enteric diseases, and reduced production efficiency [5, 6].

Among the bacterial groups commonly used as indicators of intestinal microbial status and fecal contamination are Coliforms and *Escherichia coli*. Although certain strains of *E. coli* are considered part of the normal intestinal microbiota, excessive proliferation of these bacteria may indicate an imbalance in gut microbial ecology and can increase the risk of disease occurrence [7]. In poultry production, monitoring bacterial populations using Total Aerobic Bacterial Count (TABC) analysis is widely used to assess the overall microbial load and hygienic quality of biological samples [8].

Historically, antibiotic growth promoters (AGPs) have been extensively used to control harmful intestinal bacteria and improve growth performance in broilers. However, the long-term, indiscriminate use of antibiotics has raised global concerns about antimicrobial resistance, environmental contamination, and the accumulation of drug residues in poultry products intended for human consumption [9, 10]. Consequently, considerable efforts have been directed toward identifying natural alternatives that modulate the intestinal microbiota without contributing to antimicrobial resistance.

One potential alternative is sea salt, a natural product composed primarily of sodium chloride and enriched with trace minerals such as magnesium, potassium, calcium, and other micronutrients. These minerals are known to participate in various physiological processes, including osmotic regulation, electrolyte balance, enzyme activation, and maintenance of intestinal homeostasis [11, 12]. Recent evidence suggests that dietary salt intake may influence the composition and metabolic activity of gut microbiota by altering intestinal osmolarity and microbial ecological interactions [13, 14].

The antimicrobial properties of salt have long been recognized in food preservation systems. Elevated salt concentrations can inhibit bacterial growth through osmotic stress mechanisms, resulting in reduced water activity, disruption of cellular homeostasis, plasmolysis, and impaired metabolic activity [15, 16]. Previous studies have reported that increased sodium concentrations may suppress certain bacterial populations while simultaneously promoting the proliferation of salt-tolerant microorganisms, indicating that the biological effects of salt are highly dependent on concentration and environmental conditions [12, 13].

Although the influence of high-salt diets on gut microbiota has been investigated in humans and laboratory animals, information regarding the effects of sea salt supplementation on intestinal bacterial populations in broiler chickens remains limited. Furthermore, the optimal supplementation level that influences bacterial populations without inducing adverse physiological effects has not been fully elucidated. Understanding these responses is important for developing practical nutritional strategies to improve gut health and reduce reliance on antibiotics in poultry production systems.

Therefore, the present study was conducted to evaluate the effect of sea salt supplementation through drinking water at different concentrations on Total Aerobic Bacterial Count, Coliforms, and *Escherichia coli* populations in broiler feces. The findings of this study are expected to provide scientific evidence on the potential application of sea salt as a natural feed additive to modulate intestinal microbial populations in broiler chickens.

II. METHOD

Study Location and Experimental Animals

The study was conducted on a commercial broiler farm operated by PT. Armada Jaya Mandiri uses an open-house production system. A total of 72 day-old CP 707 broiler chicks were used in this experiment. Birds were raised under standard commercial management practices and provided ad libitum access to feed and drinking water throughout the experimental period.

Experimental Design and Treatments

The experiment employed a Completely Randomized Design (CRD) consisting of four treatment groups. The control group (S0) received drinking water without sea salt supplementation, whereas the treatment groups S1, S2, and S3 received sea salt supplementation at 1 g/L, 2 g/L, and 4 g/L, respectively. The number of replicates was determined using the Federer formula, resulting in a minimum of 6 experimental units per treatment.

Sample Collection and Bacteriological Examination

Fecal samples were collected from broilers at 0, 15, and 30 days of age using sterile cloacal swabs. Immediately after collection, swabs were transferred to Stuart transport medium and maintained at refrigerated conditions during transport to the laboratory.

For bacterial enumeration, 0.1 g of fecal material was homogenized in 0.9 mL of sterile distilled water, then diluted serially to 10^{-6}. Subsequently, 0.1 mL of each diluted sample was inoculated onto culture media. Total Aerobic Bacterial Count (TABC) was determined using

Nutrient Agar (NA) through the pour plate technique, whereas Coliform and *Escherichia coli* populations were enumerated using Eosin Methylene Blue Agar (EMBA) through the spread plate technique. After incubation, bacterial colonies were counted and expressed as colony-forming units (CFU).

Variables Measured

The parameters evaluated in this study included Total Aerobic Bacterial Count (TABC), Coliform count, and *Escherichia coli* count in broiler feces. These bacterial populations were used as indicators of intestinal microbial dynamics and the potential antimicrobial effect of sea salt supplementation.

Statistical Analysis

Data were analyzed using Multivariate Analysis of Variance (MANOVA) to evaluate the effects of sea salt supplementation, sampling time, and their interaction on TABC, Coliform, and *E. coli* counts simultaneously. When significant differences were detected ($P<0.05$), Duncan's Multiple Range Test was performed to compare treatment means. Statistical analyses were conducted using IBM SPSS Statistics software.

III. RESULTS AND DISCUSSION

Effects of Sea Salt Supplementation on Fecal Bacterial Populations

The multivariate analysis demonstrated that sea salt supplementation through drinking water did not significantly affect Total Aerobic Bacterial Count (TABC), Coliform populations, or *Escherichia coli* counts in broiler feces ($P>0.05$) (Table 1). Similarly, no significant interaction between treatment and sampling time was observed, indicating that the response of bacterial populations over time was not influenced by the administered sea salt concentration. In contrast, sampling time significantly affected all bacterial parameters evaluated ($P<0.05$), highlighting the importance of age-related microbial succession in the broiler gastrointestinal tract (Table 2).

The absence of a significant treatment effect suggests that sea salt supplementation at 1–4 g/L was insufficient to induce substantial changes in intestinal bacterial populations. Although salt possesses antimicrobial properties through osmotic stress mechanisms, the concentrations applied in the present study may not have been high enough to consistently suppress bacterial growth within the complex intestinal ecosystem. Furthermore, the buffering capacity of the gastrointestinal environment and the adaptive responses of resident microbiota may have reduced the biological impact of increased sodium intake. Similar observations have been reported by Chanmuang et al. [12], who demonstrated that dietary salt altered microbial composition without necessarily causing significant reductions in total bacterial abundance.

Influence of Broiler Age on Bacterial Dynamics

Sampling time exerted a significant effect on TABC, Coliform, and *E. coli* populations, indicating that bacterial communities underwent substantial changes during broiler development. Duncan's Multiple Range Test revealed significant differences between day 0 and subsequent sampling periods, whereas bacterial counts at days 15 and 30 were generally comparable (Figure 1-3). These findings are consistent with the concept of microbial succession, whereby the gastrointestinal tract is progressively colonized by increasingly diverse microbial communities as birds mature [17].

At hatch, the intestinal microbiota is relatively simple, with limited bacterial diversity. As broilers age, continuous exposure to feed, water, litter, and the surrounding environment promotes the establishment of a more complex microbial ecosystem. Consequently, the overall bacterial population increases during the first weeks of life and gradually stabilizes as microbial communities become more structured and competitive [6, 18].

Numerical Reduction of Bacterial Counts in Moderate Sea Salt Treatments

Although treatment effects were not statistically significant, broilers receiving 1 and 2 g/L sea salt exhibited numerically lower TABC, Coliform, and *E. coli* counts than the control group at the end of the experimental period (Figures 1-3). This trend suggests that moderate levels of sea salt may exert a mild inhibitory effect on bacterial proliferation. Elevated sodium concentrations can increase osmotic pressure and alter microbial homeostasis by disrupting ion-exchange mechanisms in the intestinal environment [13].

Salt-induced osmotic stress may reduce bacterial viability by disrupting membrane integrity, impairing nutrient transport, and promoting plasmolysis under unfavorable conditions [15]. Additionally, Coliform bacteria and *E. coli* are generally sensitive to elevated salt concentrations, which may partially explain the lower bacterial counts observed in treatments S1 and S2. Nevertheless, the magnitude of these changes was insufficient to generate statistically significant differences among treatment groups.

Increased Bacterial Counts in the Highest Sea Salt Treatment

Interestingly, the highest bacterial counts were observed in broilers receiving 4 g/L sea salt. This finding suggests that excessive salt supplementation may alter the intestinal microbial environment, favoring the proliferation of salt-tolerant microorganisms. Rather than uniformly suppressing bacterial growth, high-salt exposure may shift microbial community composition and create ecological niches for specific bacterial taxa that tolerate elevated osmotic conditions [14].

Several studies have also demonstrated that excessive dietary salt intake can induce intestinal inflammation and microbial dysbiosis. Aguiar et al. [19] reported that high-salt diets promoted intestinal inflammatory responses, while Hamad et al. [14] observed reductions in beneficial lactic acid-producing bacteria following prolonged exposure to salt. Inflammatory conditions may facilitate the expansion of opportunistic microorganisms, including *E. coli*, which are frequently associated with disturbed intestinal homeostasis [20, 21].

Table 1.
Results of Multivariate Analysis of Treatment and Timing of Sea Salt Application on TABC, Coliforms, and *E. coli*.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	ALTB	10998.819	3	3666.273	,801	,498
	Coliforms	4570.833	3	1523.611	2,713	,053
	E. coli	576,597	3	192,199	,975	,410
Time	ALTB	41361.333	2	20680.667	4,516	.015*
	Coliforms	18343.083	2	9171.542	16,329	,000**
	E. coli	3615.528	2	1807,764	9,175	,000**
Treatment* Time	ALTB	8799.222	6	1466.537	,320	,924
	Coliforms	2539.917	6	423,319	,754	,609
	E. coli	395,028	6	65,838	,334	,916

Notes: *Significant (P<0.05), **Very significant (P<0.01).

Table 2.
Multiple Range Test Results of Sea Salt Application Time on TABC, Coliforms, and *E. coli*.

Time	Day 0	Day 15	Day 30
TABC	55a	92.7ab	112.8b
Coliforms	5.4a	43.9b	30.3b
Escherichia coli	2.7a	19.5b	14.6b

Note: Different superscript letters indicate significant differences (P<0.05).

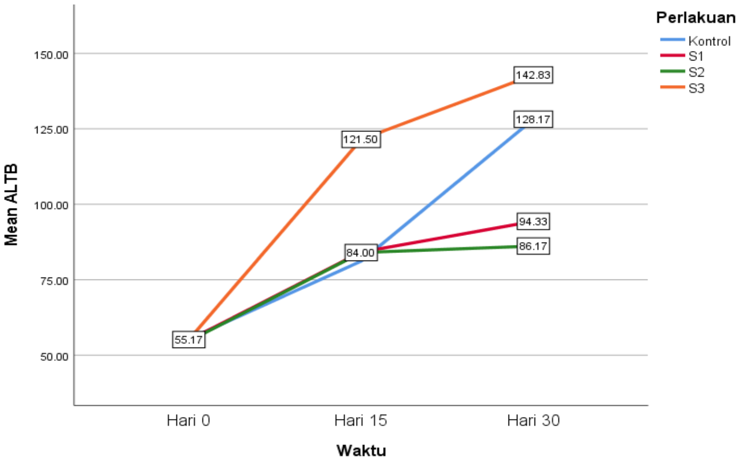

Figure 1. Graph of the average number of ALTB for 30 days

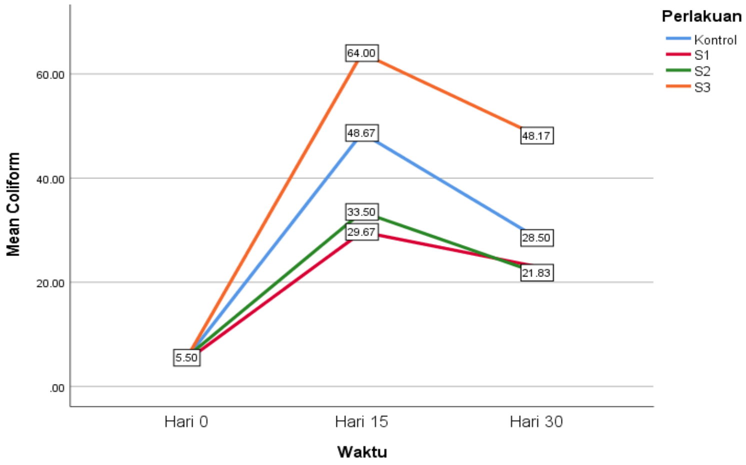

Figure 2. Graph of the average number of Coliforms for 30 days

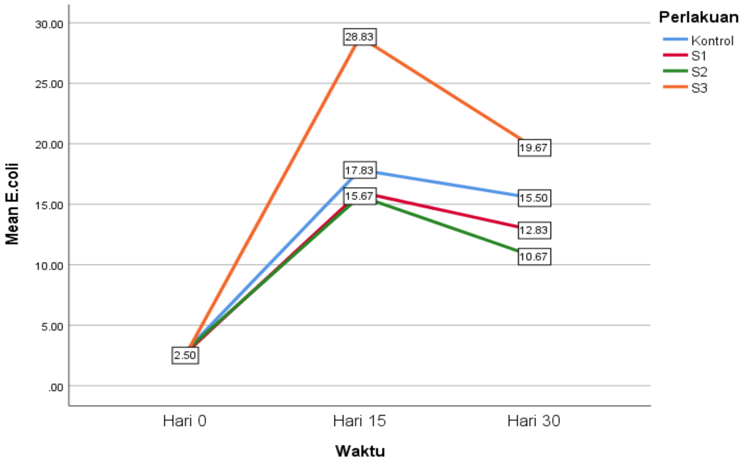

Figure 3. Graph of the average number of *E. coli* for 30 days

IV. CONCLUSION

The present study demonstrated that sea salt supplementation through drinking water at concentrations of 1, 2, and 4 g/L did not significantly affect Total Aerobic Bacterial Count (TABC), Coliform, or *Escherichia coli* populations in broiler feces. In contrast, broiler age significantly influenced all bacterial parameters, confirming that microbial succession during growth is a major determinant of intestinal bacterial dynamics. Although numerical reductions in bacterial counts were observed in broilers receiving 1 and 2 g/L sea salt, these effects were not statistically significant under the conditions of this study.

From a practical perspective, the findings suggest that sea salt supplementation alone is not an effective strategy for reducing fecal bacterial populations in broiler production. Nevertheless, moderate supplementation levels may help maintain intestinal microbial balance without adverse effects. Further studies incorporating microbiome profiling, intestinal health indicators, and production performance parameters are warranted to elucidate the broader role of sea salt as a nutritional intervention to improve gut health and support sustainable poultry production.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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