



Spice essential oils enhance broiler gut health, performance, and oxidative stability

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Abstract

Background and Objective: Optimizing gut health is critical for sustaining poultry productivity while reducing antibiotic use. This study evaluated the effects of garlic, turmeric, ginger, and cinnamon essential oils administered via drinking water on growth, intestinal morphology, oxidative stability, and gut microbial population in broiler chickens.

Methodology: A total of 200 a-day-old Arbor Acres broiler chicks weighing 38.47 ± 1.47 g were randomly distributed into five groups with control (CON), garlic essential oil (GAR), ginger essential oil (GIN), turmeric essential oil (TUR), and cinnamon essential oil (CIN), with ten replicates of four birds each, over a 42-day trial. Birds received a basal diet with essential oils in the drinking water. Growth indices, microbial counts, intestinal histomorphometry, and antioxidant status were evaluated using one-way ANOVA ($P < 0.05$).

Main Results: GAR and CIN significantly improved final body weight and villus height in duodenum compared with CON, while TUR resulted in the lowest growth performance and poorer feed efficiency. Essential oil treatments increased feed intake, particularly in GAR, without consistent improvement in feed conversion ratio. GAR reduced cecal pathogenic bacteria (coliforms and enterococci) while maintaining *Lactobacillus* populations, whereas TUR non-selectively increased both beneficial and non-beneficial microbes. GAR and GIN significantly enhanced intestinal antioxidant activity, and all treatments reduced lipid peroxidation in the duodenum and jejunum.

Conclusions: Supplementation of spice essential oils via drinking water improves gut health, oxidative stability, and growth performance in broilers, with garlic and cinnamon showing superior growth-promoting effects and ginger and garlic enhancing antioxidant capacity. These findings suggest that spice essential oils may have potential as phytogetic supplements capable of supporting broiler performance and intestinal health.

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INTRODUCTION

White meat productivity has recently improved due to the steadily growing local and global demand for it (Al-Mnaser *et al.*, 2022). The improvement of gut health and functionality is crucial to maintaining white meat productivity because it directly affects both the performance and health of animals (Kogut and Arsenault, 2016). The gut barrier, which includes mucus layers, microbiota, epithelium, underlying immune tissue, and host-derived antimicrobial compounds, interacts with dietary nutrients to maintain the necessary equilibrium that prevents the passage of toxic compounds and pathogens into the animal's system (Broom, 2018).

Irregularities in the microbiota-host interaction can lead to intestinal diseases since the microbiota is the first line of defense against enteric pathogens (Pourabedin and Zhao, 2015). Moreover, diets have been shown to influence antioxidative defense enzymes and the production of heat shock proteins and inflammatory cytokines, which in turn affect gut oxidative stability (Bacou *et al.*, 2021). Dysfunction in the intestinal barrier can result in high oxidative stress, inflammation, and apoptosis of intestinal cells (Xue *et al.*, 2020). Therefore, improving gut health through dietary supplementation, including phytogetic

additives, has gained attention for enhancing the productivity and health of broiler chickens (Prihambodo *et al.*, 2021).

Garlic (*Allium sativum* L.), turmeric (*Curcuma longa* L.), ginger (*Zingiber officinale* Roscoe), and cinnamon (*Cinnamomum zeylanicum*) have long been used as spices and natural remedies due to their potent bioactive properties. Garlic is rich in antioxidants like diallyl sulfides and allicin, which contribute to its antioxidant, anti-inflammatory, and immune-modulatory effects (Jang *et al.*, 2017). Turmeric, with its active polyphenols such as curcuminoids, has demonstrated broad anti-inflammatory, anti-cancer, and antioxidative effects (Priyanka *et al.*, 2017). Ginger contains bioactive compounds like terpenes and phenolics that exhibit antimicrobial, anticancer, antioxidant, and anti-inflammatory properties (Mao *et al.*, 2019). Cinnamon, a well-known spice, has anti-diabetic, antioxidant, anti-cancer, and anti-inflammatory properties (Weerasekera *et al.*, 2021). These spices not only have therapeutic effects but also modulate gut microbiota and oxidative stress, contributing to overall gut health in animals.

While research has highlighted the potential benefits of spice essential oils for improving gut health, most studies have focused on their dietary incorporation

rather than their effects when administered via drinking water. There is limited information on the specific effects of essential oils from garlic, turmeric, ginger, and cinnamon when provided through drinking water, especially concerning oxidative stability, intestinal mucosal development, and gut microbiota balance in broilers. This gap in knowledge presents an opportunity to explore how these essential oils could positively impact gut health and productivity in poultry. While dietary incorporation of essential oils has been studied, the effects of water administered garlic, turmeric, ginger, and cinnamon oils on broiler gut health remain poorly characterized. Specifically, no previous study has simultaneously compared these four oils via drinking water for their effects on oxidative status and intestinal histomorphometry across all three small intestinal segments. A study that explores the direct impact of these essential oils on the gut health of broilers, particularly through water supplementation, and aims to provide new insights into their potential use as nutraceutical strategies in poultry farming is novel. Unlike our previous studies, which focused on meat oxidative stability and systemic inflammatory/oxidative biomarkers, the present study specifically evaluates intestinal histomorphometry, intestinal oxidative status, and selected culturable microbial populations in broiler chickens administered spice essential oils through drinking water. The present study seeks to address this gap by examining the effects of garlic, turmeric, ginger, and cinnamon essential oils administered via drinking water on the development of intestinal mucosa, oxidative stability, and gut microbial population composition in broiler chicks.

MATERIALS AND METHODS

This study was carried out in a poultry facility (N 7035'15.85788" and E 5017'55.38588"), Teaching

and Research Farm at The Federal Polytechnic Ado-Ekiti, Ekiti State in the Southwest of Nigeria, from May to July 2023.

Ethical Consideration and Approval of the Animal Protocol

The conditions and guidelines for the use of animals and animal operations for the purposes of experiments were accepted (AP/REC/2021/004) by the research and ethics committee of The Federal Polytechnic's Animal Production Department in Ado Ekiti town, Ekiti State, Nigeria.

Processing and Evaluation of Phytogetic

Fresh and mature garlic (*A. sativum*), turmeric (*C. longa*), ginger (*Z. officinale*), and cinnamon (*C. zeylanicum*) were bought from a neighborhood spice market in Ado-Ekiti, Nigeria. The cinnamon bark, ginger rhizomes, turmeric rhizomes, and garlic cloves were manually cut into small pieces, air-dried for seven days, and then ground into a fine powder using an electric blender. As previously detailed by Dehariya *et al.* (2021), the essential oils of garlic, turmeric, ginger, and cinnamon were extracted from their respective powders and were given the names garlic essential oil, turmeric essential oil, ginger essential oil, and cinnamon essential oil. Briefly, they were peeled, diced, and blended until the mixture was thoroughly homogenized in water (40% w/v), and a uniform slurry was obtained. The homogenized mixture was transferred to a Clevenger-type apparatus for hydrodistillation. The distillation lasted for four hours to ensure maximum extraction of EOs. Once the distillation process is complete, the mixture is allowed to cool, and the EO is separated from the aqueous layer using a separating funnel. The extracted EO was dehydrated and stored in sealed glass vials below 18°C.

Experimental Animals and Design

The poultry facility consisted of a single pen (5 × 10 × 3 m; width × length × height), naturally lighted (12D:12L) with a dynamic ventilation system. The poultry pen had a 40 double-sided rearing cage system, which was evenly distributed and floored with wire mesh. The cage units were of 1 m² per unit, with 30 cm each feeding and drinking space per unit. To address the nutritional needs of broilers at the starter (1–21 days) and finisher (22–42 days) phases, an experimental basal diet was prepared following NRC recommendations (Table 1).

Thereafter, in a completely randomized design, 200 a-day-old Arbor Acres broiler chicks weighing 38.47 ± 1.47 g were randomly assigned to five treatments (control: CON; garlic essential oil: GAR; ginger essential oil: GIN; turmeric essential oil: TUR; and cinnamon essential oil: CIN) with ten replicates each and four chickens per replicate. During the starter and finisher phases of production, the birds in each treatment were offered basal feed *ad libitum* and drinking water supplemented with the different essential oils daily *ad libitum* as previously documented by Jimoh *et al.* (2023; 2024a; 2024b; 2024c). Each essential oil was emulsified before dilution; the final drinking water concentration was 3 mL of essential oil per liter of water. The fresh solution was prepared daily and offered *ad libitum* from 07:00 to 19:00, after which any leftover was measured and replaced. Dry wood shavings were spread out on the floor of each experimental unit in the experimental pen to a depth of 3.6 cm, and the temperature was maintained at $31 \pm 4^\circ\text{C}$ for 7 days, then dropped by 2°C each week until it reached $26 \pm 3^\circ\text{C}$. Throughout the 42-day trial, the hatchery's recommended vaccination schedule was strictly followed, and no medication was given.

Performance Characteristics

The feed intake and weight changes were monitored throughout the study to evaluate their

growth in a 42-day trial. Feed conversions were computed per replicate. Performance evaluation: feed intake, weight changes were monitored weekly, and feed conversion ratios (FCR) were calculated from the ratio of weight gain to feed intake. Water intake was monitored daily by subtracting the volume of leftovers from the water offered.

Sampling and Culture of Gut Microbes

A chicken from each replicate was randomly selected and slaughtered (as a representative in each of the 20 replicates per treatment) at the end of the experiment by severing the jugular vein, thereafter eviscerated, and the small intestine and caecum were taken out. The removed small intestine contents, caecum contents, and feces samples were obtained from the birds. Samples were sent to the laboratory along with intact intestine segments in an ice chest for bacteriological analysis. The standard pour plate method was utilized for the determination of the total heterotrophic count, total coliform count, total enterococci count on Enterococcus Selective Agar, and the total *Lactobacillus* count on de Man, Rogosa, and Sharpe medium.

Histomorphometry of the Intestine

From the 20 birds selected randomly per treatment above, 30 mm-long duodenum, jejunum, and ileum segments were removed as soon as they were slaughtered, rinsed with physiological saline, and fixed for 48 hours in phosphate-buffered 4% formaldehyde. According to Tomaszewska *et al.* (2021), the intestine segments were processed further for slide analysis. The obtained intestinal sections were examined for villus height, mucosa width, cryptal depth, submucosal tunical thickness, musculature coat thickness, and serosal thickness. Each variable was measured on three distinct tissue sections in ten different locations throughout each section at 10x magnification using Motic Image Plus Software,

China. Following the averaging of the measurements, each parameter's mean value for each sacrificed experimental bird was expressed.

Intestinal Oxidative Status

Each segment of the small intestine's intestinal samples was homogenized in 0.15M KCl buffer (10% w/v). Centrifuging (12,000 rpm for 45 min at 0–4°C) was done on the homogenates in a centrifuge. The methods described by Jimoh *et al.* (2021) were used to measure the supernatant's total antioxidant activity, lipid peroxidation, catalase, superoxide dismutase, and glutathione peroxidase. For inter-sample comparison, all values were adjusted to the total protein content in

each sample. The total protein was assayed with test kits obtained from Fortress Diagnostics, United Kingdom.

Total Antioxidant Activity

A reactive combination that contained 0.5 mL of Na-benzoate (10 mmol/L), 0.2 mL of H₂O₂ (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH = 7.4, prepared by mixing 19.5 mL of KH₂PO₄ (100 mmol/L) with 80.5 mL of Na₂HPO₄ (100 mmol/L), then adjusted the pH to 7.4), and 0.2 mL of Fe-EDTA complex (2 mmol/L, freshly prepared by mixing equal volumes of EDTA (2 mmol/L) and ammonium ferrous sulfate (2 mmol/L) and left to stand at 25°C for 60 min). The latter reactive mixture received ten microliters of

Table 1 Gross composition of broiler chicken ration

Composition	Starter diet	Finisher diet
Ingredient		
Maize (%)	58.50	66.50
Groundnut cake (%)	9.80	8.30
Soybean meal (%)	25.00	19.50
Fish meal (%; 65% crude protein)	3.10	2.00
Methionine (%)	0.25	0.25
Lysine (%)	0.25	0.25
Oyster shell (%)	1.50	1.50
Limestone (%)	1.10	1.20
Premix* (%)	0.25	0.25
Salt (%)	0.25	0.25
Total (%)	100.00	100.00
Nutrient analysis		
Crude protein (%)	22.99	18.58
Ether extract (%)	3.94	3.93
Crude fiber (%)	3.32	3.03
Lysine (%)	1.38	1.17
Methionine (%)	0.61	0.56
Calcium (%)	1.18	1.16
Available phosphorus (%)	0.32	0.48
Metabolizable energy (kcal/kg)	2,931.27	3,186.43

Note: * Premix composition per kg diet: vitamin A 400,000 IU, vitamin D 80,000 IU, vitamin E 40,000 ng, vitamin k3 800 mg, vitamin B1 1,000 mg, vitamin B2 6,000 mg, vitamin B6 500 mg, vitamin B12 25 mg, niacin 6,000 mg, pantothenic acid 2,000 mg, folic acid 200 mg, biotin 8 mg, manganese 300,000 g, iron 8,000 mg, zinc 20,000 g, cobalt 80 mg, iodine 400 mg, selenium 40 mg, choline 800,000 g.

the serum and was incubated at 37°C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50 mmol/L NaOH) were added, and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100°C for 10 min. The following formula was used to determine total antioxidant activity:

$$\text{Total antioxidant activity (mmol/L)} = \frac{(\text{CUA})(\text{K}-\text{A})}{(\text{K} - \text{UA})}$$

where CUA is the concentration of uric acid (mmol/L), K is the absorbance of the control (K1 – K0), A is the absorbance of the sample (A1 – A0), and UA is the absorbance of the uric acid solution (UA1 – UA0).

Lipid Peroxidation

In the reaction mixture, which had a total volume of 3 mL, there was 1 mL of TCA (0.67%) and 1 mL of serum. For 45 min, all of the test tubes were submerged in a pot of boiling water. The tubes were moved to the ice bath and centrifuged for 10 min at 2,500 rpm. By measuring the optical density of the supernatant at 532 nm, the amount of malondialdehyde (MDA) produced in each of the samples was determined.

Glutathione Peroxidase (GPx) Assay

Along with a control tube containing all the reagents except the enzyme source, 0.5 mL of 0.4 M buffer (pH 7.0), 0.2 mL of enzyme source, 0.2 mL of 2 mM GSH, and 0.1 mL of 0.2 mM H₂O₂ were added and incubated at room temperature for 10 min. The reaction was arrested by adding 0.5 mL of 10% TCA, centrifuged at 4,000 rpm for 5 min, and the GSH content in 0.5 mL of supernatant was estimated. GSH consumption was used to express the activity as g/min/mg protein.

Catalase (CAT) Assay

The test equipment included 1.0 mL of 0.059 M H₂O₂ and 1.9 mL of 0.05 M buffer (pH 7.0). The addition of a 0.1 mL enzyme source (kidney

homogenate) started the reaction. At 240 nm, the drop in absorbance was measured every 1 min for 5 min, and activity was measured in nmol of H₂O₂ decomposed per minute per mg of protein.

Superoxide Dismutase (SOD) Assay

The assay consisted of 2.1 mL of 50 mM buffer, 0.02 mL of enzyme source, and 0.86 mL of distilled water. The reaction was initiated with 0.02 mL of 10 mM pyrogallol, and the change in absorbance was monitored at 420 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in a standard assay system of 3 mL. The specific activity was expressed as units/min/mg protein.

Data Analysis

All data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) before analysis. The following one-way ANOVA model was applied to each outcome variable:

$$y_{ij} = \mu + B_i + e_{ij}$$

where y_{ij} represents the value of gut flora, enhancing performance, oxidative stability, and intestinal mucosal development measured in the i^{th} animal, μ is the overall mean for each character, B_i is the fixed effect of i^{th} spice essential oil administered, and e_{ij} is the random residual effect.

Data were tested for normality and homogeneity of variances with Levene's test and subjected to a one-way analysis of variance. Significant means were separated at $P = 0.05$, using Duncan's multiple range test using IBM SPSS 20. No adjustments for multiple comparisons were made, as each trait was analyzed separately.

RESULTS AND DISCUSSION

The performance of broilers on different essential oil treatments is shown in Table 2. The final weight of birds treated with CIN (1,495.27 g) and GAR

(1,483.81 g) was significantly ($P < 0.05$) higher than those on the control (1,467.26 g) and GIN (1,461.25 g) treatments. The birds on TUR (1,366.59 g) had the significantly ($P < 0.05$) lowest final weight. Weight gain trends show that birds on CIN (40.84 g/day) and GAR (40.48 g/day) had significantly higher values than those on TUR (37.17 g/day), suggesting that cinnamon and garlic essential oils were more effective in promoting growth performance. Feed intake of birds on essential oil-based treatments was higher than those on the control group. This increase in feed intake across the essential oil treatments, particularly GAR, indicates that these supplements may encourage greater resource consumption, which could explain the improved growth performance seen in the CIN and GAR groups. Feed conversion ratio (FCR), a key metric for resource utilization, was significantly higher ($P < 0.05$) in birds treated with TUR (2.54) compared to the control. Birds on CIN (2.32), GAR (2.45), and GIN (2.32) showed statistically ($P > 0.05$) similar FCR to those on the control, indicating comparable feed efficiency among these groups. This implies that while certain essential oils may promote higher feed intake, they do not necessarily translate into better feed conversion, highlighting a trade-off between feed consumption and growth efficiency.

Water intake also varied among treatments. Birds on CIN (268.04 mL/bird/day) and the control group (269.11 mL/bird/day) had significantly ($P < 0.05$) higher water intake compared to other treatments. Birds on GAR had a water intake of 258.72 mL/bird/day, while those on GIN (247.14 mL/bird/day) had the lowest water intake, suggesting that dietary supplements may influence the birds' hydration needs. The increased water intake in the CIN and control groups could be associated with their higher feed consumption, as increased feed intake often leads to increased water consumption. These results indicate that while cinnamon and garlic essential oils improved broiler growth performance by increasing final weight,

they were accompanied by higher feed and water intake, suggesting a trade-off between growth performance and resource utilization. The higher feed intake observed in the essential oil groups, particularly GAR, reflects a potential increase in resource consumption without a proportional increase in growth efficiency, as seen in the FCR data. Future studies should focus on economic analysis incorporating feed: gain economics to assess commercial viability. In contrast, TUR, despite promoting higher feed intake, resulted in the lowest final weight and poorer feed conversion efficiency, suggesting it may not be as effective in supporting overall growth. The differences in feed and water intake observed among treatments highlight the varying effects of dietary supplements on the metabolic processes and health of the birds (Kairalla *et al.*, 2022).

The small intestine microbial load of broiler chickens administered spice of essential oils is shown in Table 3. The small intestine total heterotrophic count in GAR (1.51 CFU/g) and TUR (1.11 CFU/g) was significantly ($P < 0.05$) higher than in CON (0.06 CFU/g) and GIN (0.05 CFU/g), with the highest count observed in the GAR and the lowest in the GIN. The total enterococci count and total coliform count in the small intestine were higher ($P < 0.05$) in TUR, compared to the CON and other treatments. The TUR and CIN administration promotes the significant ($P < 0.05$) proliferation of the small intestine total *Lactobacillus* count, compared to the CON and other treatments.

Differences in culturable bacterial counts were observed across the different regions of the gastrointestinal tract, reflecting variation in the selected microbial populations evaluated in this study. Previous studies have suggested that total coliform and enterococci counts are associated with pathogenic bacteria (Jung *et al.*, 2018), whereas total *Lactobacillus* count represents beneficial probiotic bacteria (Lutful Kabir, 2009).

Table 2 Performance characteristics of broilers on different spice essential oils

Traits	CON	GAR	GIN	TUR	CIN	P-value
Initial weight (g)	38.42 ± 2.34	37.13 ± 3.25	37.12 ± 4.34	35.70 ± 4.21	35.99 ± 3.97	0.28
Final weight (g)	1,467.26 ± 4.23 ^b	1,483.81 ± 5.24 ^a	1,461.25 ± 6.64 ^b	1,366.59 ± 10.75 ^c	1,495.27 ± 8.35 ^a	0.03
Weight gain (g/day)	39.97 ± 0.24 ^{ab}	40.48 ± 0.95 ^a	39.83 ± 0.83 ^{ab}	37.17 ± 1.47 ^b	40.84 ± 1.34 ^a	0.02
Feed intake (g/bird/day)	90.28 ± 1.95 ^b	99.04 ± 1.57 ^a	92.34 ± 3.45 ^{ab}	94.33 ± 2.95 ^a	94.69 ± 3.57 ^a	0.04
Feed conversion ratio	2.27 ± 0.10 ^b	2.45 ± 0.04 ^{ab}	2.32 ± 0.01 ^{ab}	2.54 ± 0.04 ^a	2.32 ± 0.02 ^{ab}	0.04
Average water intake (mL/bird/day)	269.11 ± 8.35 ^a	258.72 ± 6.85 ^b	247.14 ± 10.86 ^c	255.26 ± 9.78 ^b	268.04 ± 6.03 ^a	<0.00

Note: Means within the same row followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Values are presented as mean ± standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil.

Table 3 Small intestine microbial load of broilers administered spice essential oils

Traits	CON	GAR	GIN	TUR	CIN	P-value
THC × 10 ⁸ (CFU/g)	0.06 ± 0.04 ^b	1.51 ± 0.03 ^a	0.05 ± 0.02 ^b	1.11 ± 0.06 ^a	0.39 ± 0.21 ^b	0.01
TEcC × 10 ⁵ (CFU/g)	0.03 ± 0.00 ^b	0.08 ± 0.03 ^b	0.03 ± 0.00 ^b	2.46 ± 0.85 ^a	0.06 ± 0.03 ^b	0.03
TCC × 10 ⁶ (CFU/g)	2.31 ± 0.81 ^b	0.79 ± 0.36 ^c	0.04 ± 0.01 ^c	4.29 ± 0.84 ^a	2.46 ± 0.63 ^b	0.04
TLC × 10 ⁵ (CFU/g)	0.06 ± 0.02 ^b	0.36 ± 0.07 ^b	0.36 ± 0.12 ^b	2.98 ± 0.23 ^a	2.77 ± 0.18 ^a	<0.00

Note: Means within the same row followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Values are presented as mean ± standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil. THC = total heterotrophic count, TEcC = total enterococci count, TCC = total coliform count, and TLC = total *Lactobacillus* count. Number of samples per group = 20.

Dietary changes or modifications are reported as factors that can influence the gut microbial population, potentially leading to dysbiosis (Teirlynck *et al.*, 2011). In this study, the higher total heterotrophic counts observed in GAR and TUR suggest that essential oil supplementation influenced bacterial populations in the intestinal tract. Furthermore, the significant proliferation of total enterococci count, total coliform count, and total *Lactobacillus* count, particularly with TUR and CIN treatments, suggests that the spice essential oils influenced the abundance of selected culturable bacterial populations in broiler chickens.

Herbal extracts and oils are known to contain antioxidants that positively influence the intestinal microbiota, inhibiting the growth of pathogenic bacteria while stimulating the growth of beneficial, probiotic bacteria. Notably, TUR demonstrated a general amplification effect on all microbial groups studied in this trial, suggesting a non-selective promotion of both pathogenic (total enterococci count and total coliform count) and non-pathogenic/probiotic (total *Lactobacillus* count) intestinal microbes. The increase in multiple microbial counts observed with turmeric essential oil (TUR) indicates a broad effect on culturable microbial populations, implying that it may influence a broad range of culturable microbial populations evaluated in this study. While the broad amplification of microbial populations may alter the balance of the culturable bacterial populations evaluated in this study, it may also raise concerns regarding the potential overgrowth of harmful bacteria, like enterococci and coliforms, which can be pathogenic under certain conditions. The promotion of these pathogenic bacteria alongside beneficial lactobacilli could suggest that TUR does not distinguish between helpful and harmful gut microbes, leading to a broader microbial balance that may not always favor optimal gut health. In contrast, garlic essential oil was associated with higher *Lactobacillus* counts and lower counts of some microbial groups, suggesting

a potential influence on gut microbial populations in broiler chickens. This finding aligns with previous studies that demonstrated phytochemicals, like essential oils, can exert both broad-spectrum and selective effects on the gut microbes (Junaid *et al.*, 2006; Anugom and Ofongo, 2019). The non-selective microbial proliferation observed with turmeric essential oil may indicate a need for further investigation into its long-term effects on gut health. While it may contribute to a diverse gut microbiome, the unchecked proliferation of potentially harmful bacteria might require additional management strategies to ensure it doesn't negatively impact the overall health and productivity of the birds.

The effects of the spice's essential oils on the cecal microbial load of broiler chickens are shown in Table 4. The cecal total heterotrophic count and total enterococci count are reduced ($P < 0.05$) in GAR (2.16 and 0.05 CFU/g) compared to CON (4.36 and 3.58 CFU/g). While birds with TUR (4.46 and 5.48 CFU/g) had the highest total heterotrophic count, the lowest was observed in the GAR group. The cecal total coliform count in GAR (0.68 CFU/g) and GIN (1.98 CFU/g) were lower ($P < 0.05$) than in CON (4.53 CFU/g) and other treatments; while the cecal total *Lactobacillus* count in GIN (21.32 CFU/g) and TUR (19.15 CFU/g) was lower ($P < 0.05$) than in the CON (35.02 CFU/g), GAR (35.48 CFU/g) and CIN groups (34.25 CFU/g). The roles of avian caeca are very important and span across the fermentation of undigested nutrients, maintenance of a healthy gut, re-cycling of nitrogen from urine, and modulation of the gut microbes (Svihus *et al.*, 2013). As observed in this study, GAR administration was associated with reduced counts of cecal total heterotrophic, enterococci, and coliform populations. This response may indicate a potentially favorable microbial profile characterized by reduced counts of selected non-beneficial bacterial populations. In addition, the reduction in cecal *Lactobacillus* counts observed in

GIN and TUR may indicate a less favorable microbial response. This observation may reflect differences in the biological activities of the essential oils evaluated. Additionally, this discovery is consistent with a previous study by Cross *et al.* (2007) that suggested that various animal species may respond to essential oils differently when it comes to altering the gut bacterial load. Garlic essential oil reduced both pathogenic (total heterotrophic, enterococci, and coliform) and beneficial microbial counts in the cecum, while turmeric essential oil increased these counts, indicating that garlic may be associated with a healthier cecal environment by selectively reducing harmful bacteria. Despite turmeric essential oil reducing pathogenic bacteria such as coliforms and enterococci in the gut, it did not suggest enhanced growth performance as expected. This outcome can be attributed to the non-selective amplification of both pathogenic and beneficial bacteria observed in the study. While turmeric did reduce certain harmful bacteria, it also increased the population of potentially harmful microbes, such as enterococci, which can become pathogenic under these experimental conditions. This broad-spectrum effect on the gut microbes might have led to an imbalance that did not favor optimal gut health or growth performance. Furthermore, the higher feed intake observed in the TUR group did not translate into improved feed conversion efficiency, suggesting that the increased microbial load—despite a reduction in pathogenic bacteria—did not optimize nutrient utilization for growth. In comparison, garlic essential oil may indicate to selectively promote beneficial bacteria and suppressed harmful microbes, resulting in better growth performance. Thus, the lack of growth promotion with turmeric could be due to its less targeted impact on gut health, leading to a less efficient utilization of nutrients.

The fecal microbial load of broiler chickens administered with spice essential oils shows that fecal total heterotrophic count increased steadily

($P < 0.05$) in GIN (2.54 CFU/g) compared to CON (1.92 CFU/g) and other treatments (Table 5). The fecal total enterococci count in TUR (0.40 CFU/g) was reduced ($P < 0.05$) compared to CON (1.85 CFU/g), while CIN group had the highest value. The fecal total coliform counts in GAR (1.77 CFU/g) and TUR (1.22 CFU/g) were reduced ($P < 0.05$), compared to CON (2.38 CFU/g) and CIN (2.31 CFU/g), with higher coliform counts. However, the total *Lactobacillus* counts in GIN (17.83 CFU/g) and CIN (17.90 CFU/g) were lower ($P < 0.05$) than CON (33.47 CFU/g), GAR (34.75 CFU/g), and TUR (34.27 CFU/g). The digestion and absorption of nutrients depend heavily on a healthy population of pathogenic and beneficial microbiota in the gastrointestinal tract. Essential oils in particular have had a good impact on poultry productivity and can effectively prevent harmful microorganisms (Oussalah *et al.*, 2007). The proliferation of non-beneficial fecal total heterotrophic count and concurrent reduction of beneficial fecal total *Lactobacillus* count by GIN and CIN, as recorded in this study, shows that the two aforementioned essential oils were associated with more non-beneficial fecal microbes at the expense of the beneficial microbes. Furthermore, the reduction of total enterococci counts and total coliform count by TUR administration and the reduction of total coliform count by GAR administration without altering the fecal total *Lactobacillus* count population reveal the potential benefits of TUR and GAR in promoting a potentially favorable intestinal environment through suppression of selected non-beneficial bacterial populations. The roles of essential oils in promoting good gut microbe development and reduction of intestinal diseases were reported (Tiihonen *et al.*, 2010; Bento *et al.*, 2013). Fecal microbial load showed that ginger and cinnamon essential oils increased the non-beneficial heterotrophic counts, while turmeric reduced pathogenic coliform and enterococci, thus suggesting a potentially favorable microbial

Table 4 Cecal microbial load of broilers administered spice essential oils

Traits	CON	GAR	GIN	TUR	CIN	P-value
THC × 10 ⁸ (CFU/g)	4.36 ± 0.93 ^a	2.16 ± 0.78 ^b	3.53 ± 0.83 ^a	4.46 ± 1.20 ^a	3.80 ± 0.35 ^a	0.05
TEcC × 10 ⁵ (CFU/g)	3.58 ± 1.57 ^{ab}	0.05 ± 0.02 ^c	2.32 ± 0.94 ^b	5.48 ± 1.08 ^a	2.73 ± 1.00 ^b	0.03
TCC × 10 ⁶ (CFU/g)	4.53 ± 1.93 ^a	0.68 ± 0.30 ^c	1.98 ± 0.24 ^b	3.86 ± 1.05 ^a	5.20 ± 1.20 ^a	0.04
TLC × 10 ⁵ (CFU/g)	35.02 ± 2.46 ^a	35.48 ± 3.96 ^a	21.32 ± 4.26 ^b	19.15 ± 5.97 ^b	34.25 ± 3.45 ^a	0.01

Note: Means within the same row followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Values are presented as mean ± standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil. THC = total heterotrophic count, TEcC = total enterococci count, TCC = total coliform count, and TLC = total *Lactobacillus* count. Number of samples per group = 20.

Table 5 Fecal microbial load of broilers administered spice essential oils

Traits	CON	GAR	GIN	TUR	CIN	P-value
THC × 10 ⁸ (CFU/g)	1.92 ± 0.83 ^b	1.38 ± 0.62 ^b	2.54 ± 0.73 ^a	1.51 ± 0.34 ^b	2.83 ± 0.15 ^a	0.02
TEcC × 10 ⁵ (CFU/g)	1.85 ± 0.52 ^a	1.22 ± 0.74 ^a	1.30 ± 0.38 ^a	0.40 ± 0.07 ^b	1.89 ± 0.29 ^a	0.04
TCC × 10 ⁶ (CFU/g)	2.38 ± 0.47 ^a	1.77 ± 0.84 ^b	1.86 ± 0.24 ^{ab}	1.22 ± 0.58 ^b	2.31 ± 0.82 ^a	0.02
TLC × 10 ⁵ (CFU/g)	33.47 ± 8.28 ^a	34.75 ± 4.84 ^a	17.83 ± 9.36 ^b	34.27 ± 5.27 ^a	17.90 ± 7.34 ^b	0.03

Note: Means within the same row followed by different superscript letters (a, b) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Values are presented as mean ± standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil. THC = total heterotrophic count, TEcC = total enterococci count, TCC = total coliform count, and TLC = total *Lactobacillus* count. Number of samples per group = 20.

profile characterized by reduced counts of selected non-beneficial bacteria.

The effects of essential oils administration on the duodenum histomorphometry of broiler chickens are shown in Table 6. The CIN group exhibited the greatest villus height, whereas the GAR group had the lowest villus height. The duodenum villus height in CIN was higher ($P < 0.05$) than in CON, while the duodenal mucosa width in GAR was higher ($P < 0.05$) than in CON. The duodenal cryptal depth was lower ($P < 0.05$) in GAR, TUR, and CIN, compared to CON and GIN. The duodenal serosal thickness in TUR and CIN was higher ($P < 0.05$) than the values obtained for the CON. Intestine histomorphological traits affect nutritional absorption, and dietary essential oils may

have a positive effect on these traits (Mohiti-Asli and Ghanaatparast-Rashti, 2018). Cinnamon essential oil led to the highest villus height, suggesting improved nutrient absorption capability, while garlic showed the least. Garlic also showed an increase in mucosal width and a decrease in crypt depth, hinting at structural changes in the duodenum that may impact nutrient uptake.

The effects of the essential oils on the histomorphometry of the intestines of broiler chickens vary depending on whether they are applied to the ileum, jejunum, or duodenum, as was also noted in this study. Additionally, the ileum is where nutrients and water are absorbed from food, hence the changes in ileal histomorphometry of broiler chickens noted in

this study have substantial consequences. A crucial component primarily engaged in nutrition absorption is the villi, and the greater villi height results in greater absorptive and surface area, which facilitates greater nutritional absorption (Chwen *et al.*, 2013). Therefore, by implication, the reduced villus height/length due to CIN administration in this study could deter nutrient absorption and consequently the performance of broiler chickens. Furthermore, reduced mucosal width in GIN, improved cryptal depth in GAR, GIN and TUR, reduced submucosal tunical thickness in GAR, TUR and CIN, improved muscular coat thickness in GIN and improved serosal thickness in GIN and TUR of the ileum. This indicates that the essential oils exerted different effects on intestinal histomorphometry. These findings are consistent with those reported by Suicmez *et al.* (2018) who recorded increased villus height and villus surface area; and reduced crypt depth of small intestine segment of broiler chickens fed oregano and red pepper essential oils at 250 mg/kg.

Lipids, monosaccharides, peptides, amino acids, and a variety of vitamins and minerals are mostly absorbed in the jejunum (Barnes and Tappenden, 2013). Therefore, the diverse effects of spice essential oils on the histomorphometry of the jejunum of broiler chickens in this study are of importance. The jejunum histomorphometry of broiler chickens administered spice' essential oils are shown in Table 6. The GAR had the highest villus height, followed by TUR, while CIN had the lowest value. There were significant differences among means ($P < 0.05$). The widest mucosal width was observed in CON, while GAR had the narrowest width with statistical significance ($P < 0.05$). The GAR had the greatest crypt depth, followed by CIN, while TUR had the shallowest depth. Submucosal tunical thickness in CIN differed significantly ($P < 0.05$) from that of the other treatments, whereas the TUR group had the thinnest submucosal tunica. The CIN recorded the thickest muscular coat thickness, while GIN had the thinnest. There were

significant differences ($P < 0.05$) in the serosal thickness between the CON and essential oil administration, while CON had the thickest.

The ileum histomorphometry of broiler chickens administered with spice' essential oils is shown in Table 6. The ileal villus height in CIN was lower ($P < 0.05$) than in CON and other treatments. The GIN administration lowered ($P < 0.05$) the ileum mucosal width compared to CON and other treatments. The villus height was significantly higher ($P < 0.05$) in broilers administered GIN, followed by GAR, while CIN had the lowest value. Broilers administered CIN had significantly ($P < 0.05$) wider mucosal width than those on GIN. The CON and CIN resulted in the least crypt depth. Submucosal tunical thickness in CON and GIN differed significantly ($P < 0.05$) from the others. Meanwhile, the muscular coat thickness in GIN was significantly ($P < 0.05$) than other groups. For instance, the increased villus height/length in GAR and TUR suggests possible enhanced absorption of monosaccharides, lipids, amino acids, peptides, minerals, and vitamins (Barnes and Tappenden, 2013). The reduced mucosal width in GIN, elevated cryptal depth in GAR, TUR and GIN; reduction in submucosal tunical thickness in GAR, TUR and CIN; increased muscular thickness in GIN and enhanced serosal thickness in TUR in this study reflect differences in the physiological responses associated with the various essential oils. The duodenum, jejunum, and ileum form the sequential segments of the small intestine. The ileum connects to the large intestine through the ileocecal junction. The variation in the villus height, mucosal width, cryptal depth, submucosal tunical thickness, muscular coat thickness, and serosal thickness in response to the various spice essential oils used in this study further demonstrates the differential effects of the essential oils on intestinal morphology (Kamel, 2000). Garlic essential oil enhanced villus height in the jejunum, supporting better nutrient absorption.

Table 6 Duodenum, jejunum and ileum histomorphometry of broilers administered spice essential oils

Traits	CON	GAR	GIN	TUR	CIN	P-value
Duodenum						
Villus height (µm)	260.28 ± 10.84 ^b	306.83 ± 12.69 ^{ab}	312.90 ± 17.23 ^{ab}	285.79 ± 7.84 ^{ab}	318.25 ± 28.46 ^a	0.01
Mucosal width (µm)	39.04 ± 0.95 ^b	48.21 ± 2.85 ^a	38.33 ± 1.03 ^b	44.95 ± 1.13 ^{ab}	40.32 ± 1.24 ^b	0.02
Cryptal depth (µm)	128.29 ± 6.25 ^a	102.39 ± 2.85 ^b	125.25 ± 5.17 ^a	105.08 ± 4.84 ^b	97.38 ± 7.28 ^b	<0.00
Submucosal tunical thickness (µm)	22.17 ± 0.93 ^c	22.25 ± 0.79 ^c	29.70 ± 3.26 ^a	26.74 ± 2.23 ^{ab}	26.26 ± 1.96 ^b	<0.00
Muscular coat thickness (µm)	92.18 ± 5.27 ^a	61.45 ± 5.94 ^b	84.67 ± 5.95 ^a	58.85 ± 9.36 ^b	66.95 ± 7.76 ^b	<0.00
Serosal thickness (µm)	17.95 ± 6.99 ^c	19.36 ± 5.27 ^{bc}	20.85 ± 6.26 ^{abc}	24.08 ± 2.35 ^a	21.99 ± 4.84 ^{ab}	<0.00
Jejunum						
Villus height (µm)	262.37 ± 4.78 ^c	333.99 ± 8.46 ^a	286.69 ± 5.73 ^{bc}	313.61 ± 7.33 ^{ab}	249.14 ± 6.98 ^c	<0.00
Mucosal width (µm)	54.28 ± 3.89 ^a	40.87 ± 3.56 ^b	41.85 ± 4.75 ^b	41.97 ± 5.37 ^b	46.59 ± 6.89 ^b	<0.00
Cryptal depth (µm)	78.38 ± 10.53 ^c	130.07 ± 9.26 ^a	98.21 ± 5.84 ^{bc}	91.28 ± 4.85 ^{bc}	109.19 ± 6.83 ^b	<0.00
Submucosal tunical thickness (µm)	24.82 ± 1.84 ^b	24.02 ± 2.38 ^b	21.44 ± 2.58 ^b	22.44 ± 2.94 ^b	27.65 ± 1.74 ^a	<0.02
Muscular coat thickness (µm)	58.03 ± 4.83 ^b	58.03 ± 5.80 ^b	50.32 ± 5.26 ^c	60.74 ± 5.28 ^b	73.10 ± 8.26 ^a	<0.00
Serosal thickness(µm)	22.52 ± 1.35 ^a	20.10 ± 0.45 ^b	18.54 ± 2.35 ^b	19.28 ± 1.84 ^b	18.52 ± 2.59 ^b	<0.00
Ileum						
Villus height (µm)	269.37 ± 12.47 ^a	297.24 ± 9.25 ^a	301.56 ± 24.59 ^a	281.65 ± 17.35 ^a	176.88 ± 16.68 ^b	<0.00
Mucosal width (µm)	48.59 ± 1.38 ^a	49.34 ± 1.95 ^a	38.98 ± 0.99 ^b	49.84 ± 1.46 ^a	50.64 ± 2.97 ^a	0.05
Cryptal depth (µm)	88.02 ± 3.68 ^b	108.64 ± 7.36 ^a	106.30 ± 7.28 ^a	107.95 ± 8.46 ^a	89.80 ± 3.85 ^b	0.02
Submucosal tunical thickness (µm)	40.15 ± 4.68 ^a	21.92 ± 6.94 ^b	38.00 ± 3.75 ^a	20.84 ± 5.36 ^b	24.78 ± 6.38 ^b	<0.00
Muscular coat thickness (µm)	60.70 ± 9.32 ^b	58.90 ± 7.36 ^b	85.60 ± 4.78 ^a	63.70 ± 3.57 ^b	56.94 ± 4.96 ^b	<0.00
Serosal thickness (µm)	17.84 ± 1.64 ^c	19.00 ± 2.77 ^{bc}	22.10 ± 1.34 ^{ab}	22.98 ± 0.76 ^a	20.13 ± 4.28 ^{abc}	<0.00

Note: Means within the same row followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Values are presented as mean ± standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil. Number of samples per group = 20.

However, cinnamon essential oil showed lower villus height in the ileum, which could reduce absorption efficiency. The variation in histomorphometry across intestinal segments reflects the unique influence of each essential oil on the broilers' intestinal structure.

The intestinal total protein of broiler chickens treated with spice essential oils is shown in Figure 1A. CIN had the highest ($P < 0.05$) levels of total protein in the duodenum, followed by CON, which was also higher ($P < 0.05$) than GAR, GIN, and TUR. The total protein in the jejunum was highest in CON, compared to the rest of the groups, while the least ($P < 0.05$) total protein was reported in GAR and GIN. The total protein in the ileum was highest ($P < 0.05$) in GAR and CIN, compared to CON and other groups. The effects of essential oil administration on intestinal antioxidant activity of broiler chickens varied across segments of the intestine (Figure 1B). GIN had the highest ($P < 0.05$) duodenum total antioxidant activity, compared to CON and other groups. GAR, GIN, and CIN had the highest jejunal total antioxidant activity compared to CON and TUR. The GIN had the highest ($P < 0.05$) ileal total antioxidant activity, compared to CON and other groups.

Figure 1C shows the intestinal lipid peroxidation of broiler chickens administered spice essential oils. The spice essential oils reduced ($P < 0.05$) the duodenal and jejunal lipid peroxidation, compared to CON; while the essential oil administration did not affect ($P > 0.05$) the ileum lipid peroxidation. The superoxide dismutase level of the duodenum and jejunum improved ($P < 0.05$) in broilers administered GAR, GIN, TUR, and CIN, compared to CON (Figure 1D). The superoxide dismutase of the ileum improved ($P < 0.05$) in those administered GIN, TUR, and CIN, compared to CON and GAR. Figure 1E shows the intestinal glutathione peroxidase activity of broiler chickens administered spice essential oils. The duodenum glutathione peroxidase improved ($P < 0.05$) in GAR, GIN, and TUR, compared to CON

and CIN. The jejunal glutathione peroxidase improved ($P < 0.05$) in GAR and TUR, compared to CON and the other groups. While the ileal glutathione peroxidase improved ($P < 0.05$) in GAR, GIN, and CIN, compared to CON and TUR. The intestinal catalase activity varied across the intestinal segments, as shown in Figure 1F. The duodenal catalase was higher ($P < 0.05$) in GAR and TUR, compared to CON and other groups. The jejunal catalase activity was higher ($P < 0.05$) in GAR, GIN, TUR, and CIN compared to CON, while the ileal catalase activity was lower ($P < 0.05$) in GAR and CIN compared to CON and other groups.

Most biologically active compounds contained in various spices and their chemical structures have been reported to produce therapeutic effects and antioxidant activities (Yashin *et al.*, 2017). The variation in antioxidant responses observed across treatments may reflect differences in the properties of the essential oils in this study. The trend of results obtained in this study indicates that cinnamon essential oil resulted in higher protein levels in the duodenum, potentially aiding digestive enzyme activity. In contrast, garlic essential oil enhanced protein in the ileum, which may support nutrient absorption at this later stage in the gut. Ginger and turmeric essential oils were associated with higher antioxidant enzyme activities in certain intestinal segments. This suggests a protective role against oxidative stress. Garlic, ginger, and turmeric essential oils improved glutathione peroxidase activity, reflecting enhanced antioxidant protection, particularly in the jejunum and ileum. In addition, the effects of the essential oils on the duodenum, jejunum, and ileum, being different on the intestine's total antioxidant, reflect differences in the biological properties of the essential oils used and the multi-diversity effects on the various intestinal segments (Choi *et al.*, 2014; Yashin *et al.*, 2017). This observation is important because the correlation between intestinal nutritional absorptive capacity

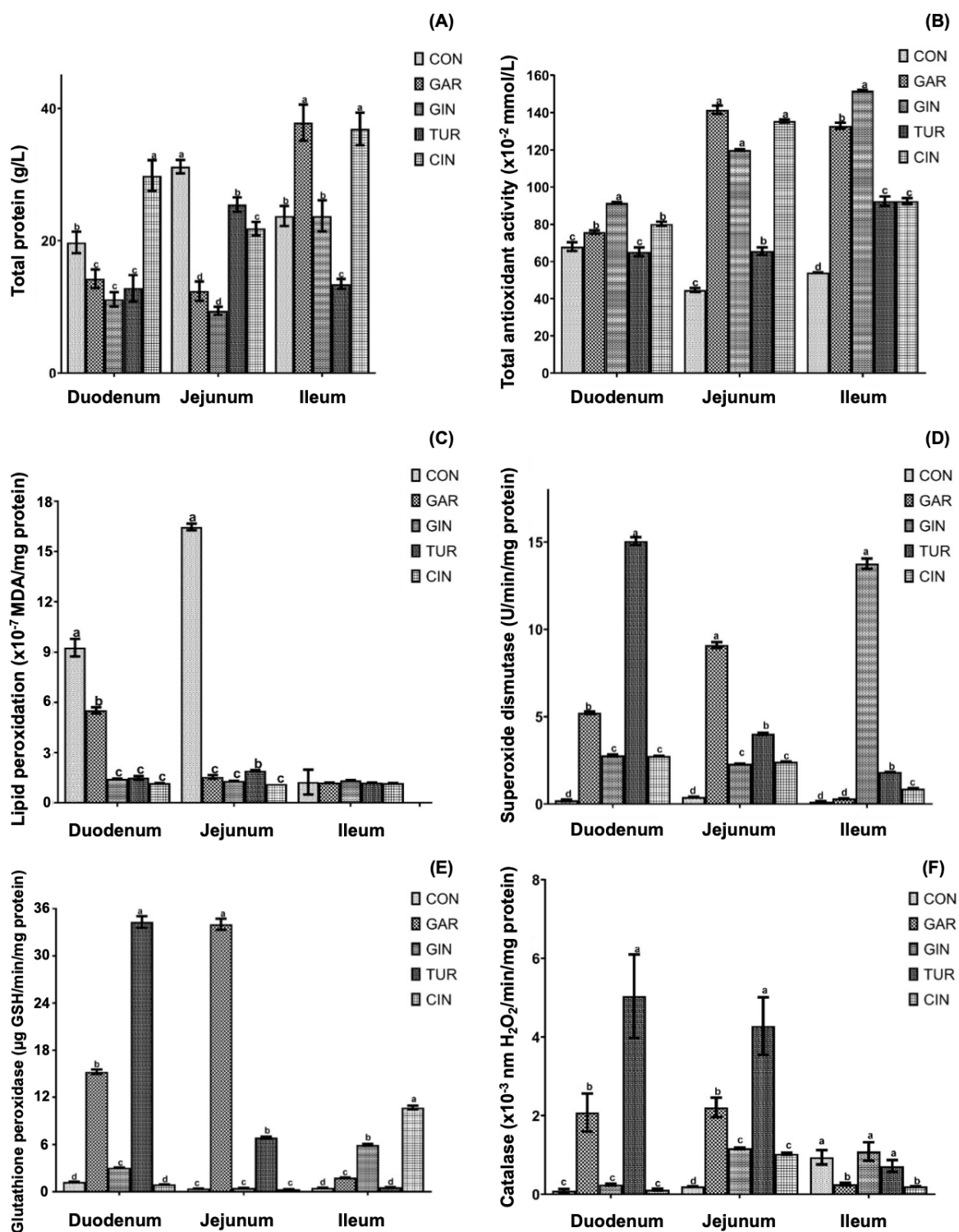


Figure 1 Intestinal protein (A), antioxidant activity (B), lipid peroxidation (C), superoxide dismutase activity (D), glutathione peroxidase activity (E), and catalase activity (F) of broilers administered spice essential oils. Different letters (a, b, c) above the bar indicate significant differences ($P < 0.05$) according to Duncan's multiple range test. Values are presented as means, and error bars represent standard deviation. CON = control, GAR = garlic essential oil, GIN = ginger essential oil, TUR = turmeric essential oil, and CIN = cinnamon essential oil. Number of samples per group = 20.

and antioxidant activity has been reported (Chedea *et al.*, 2018).

Spices and their essential oils, being rich in antioxidants (Yashin *et al.*, 2017), are proposed as preventive agents against lipid oxidation (Serafini and Peluso, 2016). This may partly explain the reduced duodenal and jejunal lipid peroxidation recorded in broiler chickens administered dietary spice essential oils. For instance, previous studies have reported that phytochemicals commonly present in spice-derived products possess antioxidant properties. The rate of lipid peroxidation is low in the ileum, hence the spice essential oils had no effects on peroxidation rates in the ileum.

The role and effectiveness of the first-line defence antioxidants, which basically include catalase, superoxide dismutase, and glutathione peroxidase, are important and indispensable in the entire defence strategy of antioxidants, especially in reference to superoxide anion radical, which is perpetually generated in normal body metabolism, particularly through the mitochondrial energy production pathway (Ighodaro and Akinloye, 2018). Consequently, the improved intestinal superoxide dismutase, glutathione peroxidase, and catalase across the duodenum, jejunum, and ileum of broiler chickens administered spice essential oils in this study further support the antioxidant potential associated with the spice essential oils evaluated in this study.

Limitations

The present study originated from the same broader experimental trial previously used in related publications by the authors; however, the datasets, analyses, endpoints, tables, figures, and interpretations reported herein are entirely distinct and have not been previously published. While the present study provides valuable insights, several limitations should be noted. The reliance on culture-based microbial enumeration,

which does not capture non-culturable or low-abundance bacterial species that may contribute to gut health. Furthermore, the chemical composition of the essential oils was not characterized in the present study, which limits direct attribution of biological responses to specific phytochemical constituents. Finally, the 42-day experimental period reflects the market age of broiler for practical responses and may not account for longer-term impacts in other birds like turkey, pullets, and cockerels on health, productivity, or microbial stability. Future work incorporating molecular microbiome analysis, essential oil compositional profiling, and extended trial durations would strengthen understanding of these relationships.

CONCLUSIONS

The study indicates that supplementing broiler drinking water with spice essential oils had distinct effects on performance, gut health, and oxidative stability. Garlic and cinnamon oils consistently improved final weight, feed intake, and villus height, indicating increased absorptive surface area and growth potential. Ginger and turmeric oils showed the greatest improvements in intestinal antioxidant activity, with turmeric additionally influencing microbial balance. All essential oils reduced lipid peroxidation in the duodenum and jejunum, reflecting antioxidant benefits. These findings suggest that garlic and cinnamon essential oils are particularly promising for improving broiler growth performance, while ginger and turmeric may serve as targeted interventions to boost antioxidant capacity. Incorporating such water-based phytogetic supplements into poultry management may contribute to improved intestinal physiological responses and oxidative stability under the conditions of this study.

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