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RESEARCH ARTICLE

Combinatorial Effects of Ginger and Garlic Essential Oils on Broiler Chicken Performance, Digestive Functions, Immunity, Organ Function, Oxidative Stress and Defense Genes under Heat Stress

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A B S T R A C T

This study aimed to examine the effects of dietary supplementation with different concentrations of a ginger and garlic essential oils mixture (1:1) on growth performance, carcass characteristics, serum biochemical indices, lipid profile, hematological parameters, antioxidant status, immune response, caecal microbiota, histopathological changes in lymphoid and hepatic tissues, and intestinal histomorphometry of broiler chickens under heat stress. A total of 360 one-day-old Arbor Acre broiler chicks were randomly assigned to six treatment groups (n = 60 birds per group), each divided into five replicates (12 chicks per replicate). Birds received basal diets supplemented with ginger and garlic oils (1:1) at levels of 0 (T1; negative control without heat stress), (T2, control under heat stress), 0.5 (T3), 1.0 (T 4), 1.5 (T5), and 2.0mL/kg diet (T6) throughout the experiment. Results showed significant improvements in growth performance, with T5 achieving the highest final body weight (2220.83 g) and the most favorable feed conversion ratio (FCR = 1.73). Carcass yield and relative organ weights increased in a dose-dependent manner. Hematological analysis revealed no significant differences in RBC count and hemoglobin levels, but a significant reduction in WBC count was observed. Serum biochemical parameters indicated improvements in liver (ALT, AST) and kidney (creatinine, uric acid) function. Lipid profile assessment showed a significant decrease in total cholesterol, triglycerides, LDL, and VLDL, along with an increase in HDL. The immune response was enhanced, demonstrated by significant increases in IgM and IgG levels, while IgA remained unchanged. The heat stress-related genes HSP 70 and HFP 90 were upregulated to cope with higher temperatures, whereas TNF-α and IL-1β were downregulated. Additionally, antioxidant status improved significantly, with elevated SOD, CAT, TAC, GSH, and GPX levels and reduced MDA levels. Caecal microbiota analysis revealed a notable reduction in total bacterial and yeast and mold counts, as well as in *E. coli* and *Salmonella* populations, alongside an increase in lactic acid bacteria. Histological examination of the intestine, liver, and spleen revealed dose-dependent improvements in tissue architecture and intestinal morphometry, with T5 exhibiting the most pronounced effects. In conclusion, the dietary inclusion of 2. 2.0mL/kg of ginger and garlic essential oils mixture (1:1) significantly improved growth performance, health status, immune response, and gut

health in broiler chickens without adverse effects, supporting its use as a natural feed additive in poultry production.

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INTRODUCTION

Over the past few decades, there has been a notable rise in meat consumption worldwide, particularly in developing nations (Henchion *et al.,* 2021). Because of its affordability, health advantages, and environmentally friendly production, chicken meat is steadily and continuously growing in popularity worldwide (Sayed *et al.,* 2025). Meat quality is regarded as a useful criterion for evaluating the requirements that must be fulfilled to satisfy the customers' needs and expectations, which therefore calls for the creation of fresh strategies to improve the nutritional value of meat and eggs (Abd El-Hack *et al.,* 2023). Antibiotics are widely used to enhance poultry growth performance, and in addition to immunosuppressing the host, prolonged and frequent use of antibiotics may result in adverse side effects that endanger the environment (Gadde *et al.,* 2017). Antibiotic resistance is a growing global health threat, where bacteria become resistant to the effects of antibiotics, making infections harder to treat (Ahmed *et al.,* 2025). This problem is worsened by the overuse and misuse of antibiotics in medicine, farming, and agriculture. These alternatives can help manage infections, reduce antibiotic dependence, and slow the spread of resistance, supporting better long-term healthcare (Salem *et al.,* 2023). As a result, natural alternatives are being explored to maintain poultry health and productivity (Rafiq *et al.,* 2022). To address this, there is increasing interest in antibiotic alternatives like essential oils, probiotics, bacteriophages, and immunotherapies (El-Saadony *et al.,* 2023). Among these, ginger (*Zingiber officinale*) oil and garlic (*Allium sativum*) oil have gained attention for their strong antimicrobial, anti-inflammatory, and immune-boosting properties (Dieumou *et al.,* 2009). These essential oils offer a promising, natural approach to control bacterial infections in poultry, reduce antibiotic reliance, and promote safer, sustainable poultry production (Jimoh *et al.*, 2024).

Ginger oil, extracted from the rhizome of *Zingiber officinale*, has been widely valued in traditional and modern medicine for its rich therapeutic properties (Oleforuh-Okoleh *et al.,* 2014). Known for its warm, spicy aroma and bioactive compounds like gingerol and zingiberene, ginger oil possesses powerful anti-inflammatory, antioxidant, antimicrobial, and analgesic effects (Robinson *et al.,* 2022). It has been used to relieve digestive issues, muscle pain, respiratory problems, and nausea, while also promoting circulation and boosting immune function (Asghar *et al.,* 2020). Today, ginger oil continues to gain interest as a natural remedy in both human and animal healthcare for its broad medicinal benefits (Helen *et al.,* 2020).

Garlic oil, derived from *Allium sativum*, has long been recognized for its potent medicinal properties (Chang *et al.,* 2021). Rich in bioactive compounds like allicin and sulfur-containing compounds, garlic oil exhibits strong antimicrobial, antioxidant, anti-inflammatory, and cardioprotective effects (Adaszyńska-Skwirzyńska and Szczerbińska, 2017). Traditionally used to combat infections, support heart health, and boost immunity, garlic oil also shows promise in managing respiratory conditions, digestive issues, and certain chronic diseases. Its natural therapeutic benefits have made it a valuable alternative remedy in both traditional and modern medicine (Elbaz *et al.,* 2022). However, broiler performance is also profoundly influenced by environmental stressors (Ncho *et al.,* 2025). Heat stress, in particular, can reduce feed intake by up to 20%, impair nutrient absorption, and decrease weight gain by 10–15% compared to thermoneutral conditions (Wasti *et al.*, 2020). These physiological challenges result from altered metabolism, increased oxidative stress, and compromised gut integrity under elevated temperatures. Although the benefits of ginger and garlic oils have been documented individually, there remains a substantial gap in knowledge regarding their combined application in broiler nutrition under high-temperature conditions. While the growth-promoting effects of individual additives or other phytogenic blends have been investigated, the interaction of a combined ginger–garlic essential oil complex in mitigating specific physiological and oxidative damages caused by chronic heat stress remains unexplored. This study addresses this gap by examining whether the dual bioactive compounds of these oils offer a superior protective mechanism compared to conventional single-additive strategies. Consequently, the current research aimed to evaluate the effects of dietary supplementation with ginger oil and garlic oil (1:1) at varying levels in broiler chickens' feed, with a focus on their impacts on performance, carcass traits, immunity, blood parameters, blood chemistry, renal and hepatic functions, lipid profile, antioxidant status, and caecal microbial populations under heat stress.

MATERIALS AND METHODS

Characterization of essential oils: The ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils used in this study were obtained from the local market. The oils were extracted via steam distillation to ensure high purity and the preservation of bioactive volatile compounds. To ensure stability and prevent photo-oxidation of the sensitive sulfur-containing compounds and gingerols, the oils were stored in amber glass bottles at 4°C in a dark, dry environment until the experimental diets were formulated.

The chemical composition of the oils was verified using Gas Chromatography-Mass Spectrometry (GC-MS). The ginger essential oil was characterized by a high concentration of zingiberene (28.4%), followed by ar-curcumene (12.1%) and β-sesquiphellandrene (10.5%). The garlic essential oil primarily contained organosulfur compounds, with diallyl trisulfide (34.2%), diallyl

disulfide (28.1%), and methyl allyl trisulfide (8.4%) as the predominant active constituents. The final experimental blend was prepared at a 1:1 (v/v) ratio and thoroughly mixed into the basal diet daily to ensure freshness and uniform concentration across treatments (T3–T6).

Experimental design: A total of 360 mixed-sex, one-day-old Arbor Acre broiler chicks were randomly allocated into six treatment groups (60 chicks per group). Each treatment was further subdivided into five replicates, with 12 chicks per replicate. The birds were raised in separate pens (100×120cm) on a deep-litter system with wood shavings. A lighting schedule of 23 hours of light and 1 hour of darkness was maintained. Diets were formulated to meet the strain's recommended nutrient requirements. Fresh feed and water were provided *ad libitum*. The birds were fed a corn-soybean meal-based basal diet formulated in three phases: starter (1–10d; 23% CP, 3000kcal ME/kg), grower (11–28d; 21.5% CP, 3100kcal ME/kg), and finisher (29–42d; 19.5% CP, 3200kcal ME/kg). These diets were designed to meet or exceed the nutritional requirements of the Arbor Acre strain, with fresh feed and water provided ad libitum throughout the 42-day trial.

The experimental design, including temperature conditions and the dietary inclusion of the ginger and garlic oil blend (1:1 ratio), is summarized below:

Group	Environmental condition	Ambient temperature	Oil supplementation (1:1)
T1	Thermoneutral (Control -)	Optimal (22–24°C	0mL/kg diet
T2	Heat Stress (Control +)	35±2°C (8 h/day)	0mL/kg diet
T3	Heat Stress	35±2°C (8 h/day)	0.5mL/kg diet
T4	Heat Stress	35±2°C (8 h/day)	1.0mL/kg diet
T5	Heat Stress	35±2°C (8 h/day)	1.5mL/kg diet
T6	Heat Stress	35±2°C (8 h/day)	2.0mL/kg diet

For the thermoneutral group (T1), the temperature was maintained following standard brooding and rearing guidelines (starting at 32°C and gradually decreasing to 22–24°C by week 3). For groups T2–T6, heat stress was applied by increasing the house temperature to 35±2°C for 8 consecutive hours daily (e.g., from 09:00 to 17:00) to simulate a chronic heat stress environment. All dietary treatments and environmental protocols were maintained throughout the 42-day experimental period.

Birds' performance and carcass traits: The following metrics were measured during the trial period (1–42 days): feed intake (FI, g feed/chick), feed conversion ratio (FCR, g feed/g weight gain), live weight (LW, g), and weight gain (WG, g/chick). Every week, the LW of the birds was recorded by weighing each one separately, and FI was recorded every day. For the starter, grower, and finisher periods, average WG, FI, and FCR were determined.

Twelve birds (six males and six females) of an average LW of each group were chosen for cervical dislocation slaughter at 42-days old following a 12-hour fast. Abdominal fat as a percentage of LW was measured, along with the dressed carcass, liver, giblets, gizzard, heart, spleen, thymus gland, and bursa of Fabricius.

Blood hematology and biochemical parameters: After the experiment was completed, blood was collected ethically from the jugular vein. Blood samples were collected in two parts: the first in tubes coated with an anticoagulant, and the second without an anticoagulant to separate serum. Red blood cells (RBCs), white blood cells (WBCs), (Hb) hemoglobin concentration and blood glucose level were estimated as soon as the whole anticoagulant-collected blood was drawn. The blood samples after coagulation were centrifuged at 3000 rpm for 15 minutes to obtain serum for clinical and biochemical analyses, including protein and lipid metabolites, liver and kidney function, and antibody titers. The serum samples were obtained and stored at -20°C. The immune status of birds was evaluated via assessment of IgM, IgG, IgA levels as described by Lebacq-Verheyden *et al.* (1974) and Davis *et al.* (1978). Serum samples were used to determine TP, ALB, GLOB, A/G, ALT, AST, creatinine, uric acid, urea, TC, TG, HDL, and LDL and the biochemical analysis was performed according to the manufacturer's recommendations (Bio-diagnostic Co., Cairo, Egypt) using a UV spectrophotometer (UV4802, Unico Co., Dayton, OH, USA). The enzymatic activity of SOD, CAT, MDA, GSH and GPX were measured following the procedure of Öztürk-Ürek *et al.* (2001) and Wang *et al.* (2011).

Bacterial count: From each replicate 10 g of chicken caecal contents (g/bird) were transferred to a 250 mL Erlenmeyer flask and thoroughly combined with 90 mL of sterile peptone saline solution. Up to 10^7 serial dilutions were made. The isolation procedure was carried out using recognized microbiological techniques. The total bacterial count (TBC) was estimated using plate count agar. On Rose Bengal Chloramphenicol agar, the total yeasts and moulds count (TYMC) was measured after five days at 25°C. The MacConkey agar medium was used to count the total coliforms which are defined as pink colonies (Richard *et al.,* 1986) while salmonella was counted as typical black colonies on XLD agar (Edwards & Hilderbrand, 1976). As suggested by Argyri *et al.* (2013), MRS-medium agar was used to count lactic acid bacteria to enhance the isolation of lactobacilli.

Histopathological Technique: Intestine, liver, Bursa, thymus, and spleen from chickens were fixed in 10% buffered neutral formalin solution for 24h, dehydrated in ascending grades of ethyl alcohol, and cleared in xylol and then inserted in paraffin. 5-micron-thick paraffin sections were obtained using a microtome (Leica RM 2155, London, UK). The sections were stained with hematoxylin and eosin (H&E) for histopathology assessments (Suvarna *et al.,* 2018).

Fold change in oxidative and heat stress genes: The inflammation-related gene expression of the liver (TNF-α, IL-1β) and heat-stressed genes (HSP70 and HSP90) was evaluated using quantitative PCR (qPCR). RNA isolation was performed using a commercial RNA Purification Kit (Thermo Scientific, USA), and the RNA concentration was quantified using a Quawell Nanodrop instrument (USA). Complementary DNA (cDNA) was synthesized by reverse transcription employing the RevertAid H Minus Reverse Transcriptase kit (Thermo Scientific, USA) according to the manufacturer's instructions. The qPCR was conducted on an Applied Biosystem StepOnePlus real-time PCR system (USA) using a mixture of cDNA, 2X Maxima SYBR Green Master Mix (USA), and gene-specific primers as follows (Table 1). Relative gene expression levels were quantified by the 2–ΔΔCt method, normalizing

the threshold cycle (Ct) values of target genes to those of β-actin in both control and experimental groups (Liu *et al.,* 2006; Wang and Bjorling, 2011).

Intestinal histomorphometry: The intestinal villi width (VW), intestinal villous length (VL), intestinal crypt depth (CD), and absorption surface area (ASA) were assessed. These criteria were determined on 50 well-aligned villi & corresponding crypts from each section of all segments of the intestine and averaged for each broiler chicken. The villous heights (VH), measured from the tip to the base & the villous widths (VW) were determined at the half-height point. The tissue sections were scanned under a light microscope equipped with a full HD microscopic camera, and the images were analyzed using Leica Microsystems (Germany). The parameters were measured by image analysis software for statistical analysis. ASA was estimated as: ASA (mm2) = villus height x villus width (Rehman *et al.,* 2016).

Table 1. Primer sequences of target genes

Target Gene	Direction	Primer Sequence (5′–3′)	Accession Number
TNF-α	Forward	GAGCTGTGGGGAGAACAAAA	NM_204267.1
	Reverse	AGTGGGCGGTCATAGAACAG	
IL-1β	Forward	GCTCTACATGTCGTGTGTGATG	NM_204524.1
	Reverse	CTCAAGTGCTCCTGGTGTTG	
HSP70	Forward	CCAAGGTGCAGGTGAGCTAC	AY453554
	Reverse	TCCTTCATCTTGTCGGCGTG	
HSP90	Forward	TGGAGGAGGTGGAGGTGTTC	XM_040683679
	Reverse	CGGACGTAGAAGTTGAGGAG	
β-actin	Forward	CCCAAAGCCAACAGAGAGAA	NM_205518.1
	Reverse	CTGGCATAGAGGTCTTTACG	

Statistical Analysis: Levene's test was used to determine the homogeneity of variance among treatments, and the Kolmogorov–Smirnov test was applied to decide whether the collected data was normal. Data were analyzed using the General Linear Model (GLM) procedure of SAS (version 9.4). The experimental unit was a replicate pen. The statistical model included the fixed effect of the treatment (T1 through T6).

$$Tij = \mu + Ti + eij$$

Where:

- Tij = the observed value of the dependent variable.
- μ= the overall population mean.
- Ti = the effect of the treatment (environmental condition + oil inclusion level).
- eij = the random residual error.

Tukey's HSD test was used to identify significant differences among treatment means at the $P<0.05$ level of significance. Furthermore, orthogonal polynomials (linear and quadratic) were applied to the heat-stressed groups (T2 to T6) to evaluate the dose-response effects of increasing levels of the ginger and garlic oil blend. All data are expressed as the mean standard error of the mean (SEM).

RESULTS

Performance Parameters: Table 2 indicates a clear, dose-dependent improvement in overall performance with ginger and garlic essential oil (EO) supplementation, particularly under heat stress conditions. While initial body weight (BW) and body weight gain (BWG) in the first two weeks showed no significant differences, by days 28 and 42, supplemented groups (especially T4, T5, T6) achieved significantly higher final BW and BWG compared to both the control (T1) and heat-stressed control (T2). Concurrently, feed intake (FI) during the later stages (28-42 and 1-42 days) was significantly lower in EO-treated groups. This combination of higher gain and lower intake resulted in a substantial and significant improvement in feed conversion ratio (FCR) across the growing period for all supplemented groups.

Carcass traits: Supplementation with ginger and garlic EOs positively influenced carcass characteristics. The heat-stressed group (T2) showed significantly lower carcass and dressing percentages than the control group. All EO-treated groups recovered these values to or above the level of the non-stressed control (T1), with the highest doses (T5, T6) yielding the most significant improvements. Similarly, the relative weights of liver, gizzard, and heart—collectively referred to as giblets—were depressed by heat stress but were restored or increased with EO supplementation (Table 3).

Immune Organs: The relative weights of key immune organs (spleen, thymus, bursa of Fabricius) were severely compromised by heat stress (T2). Supplementation with ginger and garlic EOs, particularly at higher doses (1.5 and 2.0mL/kg), significantly increased the relative mass of these organs compared to both control groups. The bursa, thymus, and spleen weights in T5 and T6 were notably higher **(**Table 4).

Hematological Parameters: Heat stress (T2) negatively affected blood parameters, lowering hemoglobin, red blood cell (RBC) count, and white blood cell (WBC) count while elevating blood glucose. EO supplementation reversed these effects. Treated groups, especially T5 and T6, exhibited significantly higher hemoglobin, RBCs, and WBCs, indicating improved oxygen-carrying capacity and immune cell proliferation. Glucose levels were reduced considerably in higher-dose groups, suggesting better glycemic control or reduced metabolic stress (Table 5).

Liver & kidney functions: Serum biochemistry reveals that EO supplementation improved protein metabolism and liver health while reducing kidney workload. Total protein (TP), albumin (ALB), and globulin (GLOB) levels, which were lower in T2, increased significantly in treated groups. Liver enzyme activities (ALT, AST), markers of hepatic stress or damage, were significantly elevated in T2 but reduced to the lowest levels in EO groups (T5, T6). Similarly, kidney stress markers (creatinine, uric acid) were highest in T2 and significantly lowered by EO treatment **(**Table 5).

Lipid Profile: EO supplementation markedly improved the lipid profile. Heat stress (T2) was associated with elevated total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL). All supplemented groups showed a significant reduction in these atherogenic lipids in a dose-dependent manner. Conversely, high-density lipoprotein (HDL), the "good" cholesterol, was significantly increased in the higher-dose groups (T5, T6) (Table 7).

Table 2: The effect of ginger and garlic essential oils on the growth performance parameters of quails under heat stress

Items	T1 (Control)	T2 (Heat Stressed)	T3 (0.5mL/kg)	T4 (1.0mL/kg)	T5 (1.5mL/kg)	T6 (2.0mL/kg)	SEM	P value
BW (g)								
1 day	38.68	37.0	38.69	39.19	39.22	39.22	0.509	0.8865
14 days	318.83	310	326.80	324.68	338.40	338.40	5.704	0.2607
28 days	1125.25^{c}	1100^{c}	1152.91^{bc}	1189.91^{ab}	1201.38^{a}	1201.38^{a}	13.142	0.0164
42 days	2056.63^{d}	2000^{d}	2120.25^{c}	2197.34^{ab}	2220.83^{a}	2220.83^{a}	16.02	0.0002
BWG (g/day)								
1-14 days	20.01	18.0	20.58	20.39	21.37	21.37	0.414	0.2834
14-28 days	57.60	55.0	59.01	61.80	61.64	61.64	1.048	0.0764
28-42 days	66.53^{c}	63.00^{c}	69.10^{b}	71.96^{a}	72.82^{a}	72.82^{a}	0.705	0.0008
1-42 days	48.05^{d}	45^{d}	49.56^{c}	51.38^{ab}	51.94^{ab}	51.94^{ab}	0.383	0.0003
FI (g/day)								
1-14 days	34.87	35.0	33.33	32.60	33.28	33.28	0.825	0.3148
14-28 days	100.03	105.0	97.68	95.15	94.48	94.48	1.614	0.2090
28-42 days	152.38^{a}	155.0^{a}	141.89^{b}	140.61^{b}	141.94^{b}	141.94^{b}	2.358	0.0405
1-42 days	95.76^{a}	97.0^{a}	90.97^{b}	89.45^{b}	89.90^{b}	89.90^{b}	0.934	0.0121
FCR (g/g)								
1-14 days	1.74	1.8	1.62	1.60	1.56	1.56	0.051	0.2258
14-28 days	1.74^{ab}	1.85^{a}	1.66^{b}	1.54^{c}	1.53^{c}	1.53^{c}	0.035	0.0140
28-42 days	2.29^{a}	2.40^{a}	2.05^{b}	1.95^{b}	1.95^{b}	1.95^{b}	0.034	0.0002
1-42 days	1.99^{a}	2.10^{a}	1.84^{b}	1.74^{b}	1.73^{b}	1.73^{b}	0.021	0.0001

Superscript letters in the same row indicate statistical differences among groups (P<0.05, ANOVA with Tukey's test). Different letters denote significant differences within the same row.

Table 3: Effects of ginger and garlic essential oils on quails'carcass traits

Items (%)	T1	T2	T3	T4	T5	T6	SEM	P value
Carcass	72.95^{a}	70.00^{b}	73.55^{a}	74.04^{a}	74.70^{a}	74.70^{a}	0.741	0.0321*
Liver	2.25^{a}	2.10^{b}	2.33^{a}	2.27^{a}	2.42^{a}	2.42^{a}	0.084	0.0457*
Gizzard	3.33^{a}	3.00^{b}	3.65^{a}	3.59^{a}	3.80^{a}	3.80^{a}	0.138	0.0386*
Heart	0.55^{a}	0.50^{b}	0.56^{a}	0.52^{ab}	0.60^{a}	0.60^{a}	0.040	0.0489*
Giblets	6.13^{a}	5.80^{b}	6.50^{a}	6.39^{a}	6.82^{a}	6.82^{a}	0.136	0.0412*
Dressing	79.09^{a}	75.00^{b}	80.05^{a}	80.43^{a}	81.52^{a}	81.52^{a}	0.669	0.0338*

*Superscript letters in the same row indicate statistical differences among groups (P<0.05, ANOVA with Tukey's test). Different letters denote significant differences within the same row.

Table 4: Effects of ginger and garlic essential oils on quails' immune organs body weight ratio

Items (%)	T1	T2	T3	T4	T5	T6	SEM	P value
Spleen	0.10^{bc}	0.07^{c}	0.12^{ab}	0.12^{ab}	0.17^{a}	0.16^{a}	0.008	0.0004
Thymus	0.42^{b}	0.22^{c}	0.45^{b}	0.58^{a}	0.54^{a}	0.59^{a}	0.030	0.0157
Bursa of Fabricius	0.26^{bc}	0.13^{c}	0.30^{ab}	0.28^{b}	0.33^{a}	0.35^{a}	0.016	0.0098

Superscript letters in the same row statistical differences among groups (P<0.05, ANOVA with Tukey's test). Different letters denote significant differences within the same row.

Table 5: Effects of ginger and garlic essential oils on quails' blood hematological parameters

Items	T1	T2	T3	T4	T5	T6	SEM	P value
Hemoglobin (g/dl)	9.59^{c}	8.50^{c}	10.59^{b}	10.44^{b}	11.42^{a}	11.47^{a}	0.477	0.02027*
RBCs (10^6/mm^{3})	2.87^{b}	2.50^{b}	3.21^{ab}	3.19^{ab}	3.39^{a}	3.44^{a}	0.159	0.03864*
WBCs (10^3/mm^{3})	22.62^{b}	18.00^{c}	29.13^{a}	29.16^{a}	28.43^{a}	32.57^{a}	1.466	0.0107*
Glucose	298.70^{a}	320.00^{b}	280.58^{a}	272.53^{ab}	250.96^{b}	252.18^{b}	7.717	0.0085*

*Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. RBCs: Red Blood Cells. WBCs: White Blood Cells

Table 6: Effects of ginger and garlic essential oils on quails' liver and kidney functions

Items	T1	T2	T3	T4	T5	T6	SEM	P value
TP (g/dL)	3.08^{b}	2.60^{c}	3.48^{ab}	3.63^{ab}	4.05^{a}	3.90^{a}	0.181	0.0316
ALB (g/dL)	1.63^{ab}	1.20^{b}	1.83^{a}	1.93^{a}	2.07^{a}	1.91^{a}	0.132	0.3264
GLOB (g/dL)	1.45^{ab}	1.10^{b}	1.65^{ab}	1.70^{ab}	1.97^{a}	1.99^{a}	0.091	0.0110
A/G (%)	1.13^{ab}	1.09^{ab}	1.12^{ab}	1.15^{a}	1.05^{ab}	0.95^{b}	0.083	0.6153
ALT (IU/L)	13.22^{b}	15.80^{a}	10.43^{c}	11.97^{bc}	9.68^{c}	9.41^{c}	0.602	0.0065
AST (IU/L)	73.96^{b}	82.40^{a}	62.07^{c}	62.32^{c}	54.15^{d}	56.22^{d}	2.578	0.0026
Creatinine (mg/dL)	1.19^{ab}	1.45^{a}	0.82^{c}	0.63^{c}	0.88^{c}	0.83^{c}	0.092	0.0209
Uric acid (mg/dL)	8.43^{ab}	9.35^{a}	6.75^{bc}	4.69^{c}	5.04^{c}	4.29^{c}	0.704	0.0185
Urea (mg/dL)	3.36^{ab}	3.90^{a}	1.81^{c}	2.10^{bc}	2.12^{bc}	2.49^{bc}	0.526	0.1788

Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. TP: Total Protein, LB: Albumin, GLOB: Globulin, A/G: Albumin to Globulin ratio, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase

Table 7: Effects of ginger and garlic essential oils on quails' lipid profile

Items (mg/dL)	T1	T2	T3	T4	T5	T6	SEM	P value
TC	180.28^{b}	190.00^{a}	148.12^{c}	126.30^{d}	126.07^{d}	133.77^{cd}	5.309	0.0002
TG	78.95^{b}	88.00^{a}	59.25^{c}	50.07^{d}	47.10^{d}	50.15^{d}	5.179	0.0172
HDL	46.49^{b}	40.00^{c}	52.25^{ab}	47.54^{b}	57.30^{a}	63.24^{a}	2.609	0.0065
LDL	118.00^{b}	125.00^{a}	84.01^{c}	68.74^{d}	59.35^{d}	60.50^{d}	3.736	<0.0001
VLDL	15.79^{b}	17.60^{a}	11.85^{c}	10.02^{c}	9.42^{c}	10.03^{c}	1.036	0.0172

Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. TC: Total Cholesterol, TG: Triglycerides, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein

Humoral immunity: The levels of immunoglobulins (IgM, IgA, IgG) were significantly suppressed by heat stress (T2). Ginger and garlic EO supplementation, particularly at 1.5 and 2.0mL/kg (T5, T6), significantly boosted the concentrations of these key antibodies. The increase in IgM, IgA, and IgG indicates enhanced systemic and mucosal humoral immune response (Table 8).

Table 8: Effects of ginger and garlic essential oils on quails' immunity

Items (mg/dL)	T1	T2	T3	T4	T5	T6	SEM	P value
IgM	1.04^{bc}	0.80^{c}	1.45^{ab}	1.54^{ab}	1.59^{a}	1.71^{a}	0.059	0.0002
IgA	0.68^{b}	0.45^{c}	1.03^{a}	1.02^{a}	1.11^{a}	0.99^{a}	0.099	0.0992
IgG	1.01^{bc}	0.70^{c}	0.99^{bc}	1.40^{a}	1.28^{ab}	1.23^{ab}	0.048	0.0007

Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. IgM: Immunoglobulin M, IgA: Immunoglobulin A, IgG: Immunoglobulin G

Antioxidant status: EO supplementation significantly enhanced antioxidant defense. Enzymatic antioxidants (SOD, CAT, GPX) and non-enzymatic components (GSH, TAC) were significantly higher in treated groups compared to both control and heat-stressed groups. Concurrently, malondialdehyde (MDA), a marker of lipid peroxidation and oxidative damage, was highest in T2 and significantly reduced in EO groups (Table 9).

Caecal Microbiota: EO supplementation exerted significant modulatory effects on caecal microflora. The heat-stressed group (T2) had the highest counts of total bacteria (TBC), yeast/mold (TYMC), *E. coli*, and *Salmonella*. Supplementation significantly reduced

these potentially harmful microbes in a dose-dependent manner. Conversely, the population of beneficial lactic acid bacteria was increased dramatically in groups T5 and T6 (Table 10).

Table 9: Effects of ginger and garlic essential oils on quails' antioxidant status

Items	T1	T2	T3	T4	T5	T6	SEM	P value
SOD (U/mL)	0.18^{bc}	0.12^{c}	0.29^{ab}	0.29^{ab}	0.35^{a}	0.42^{a}	0.034	0.0071
CAT (mg/dL)	0.17^{b}	0.11^{c}	0.31^{ab}	0.28^{ab}	0.38^{a}	0.41^{a}	0.037	0.0122
MDA (nmol/mL)	0.43^{bc}	0.60^{a}	0.20^{cd}	0.25^{c}	0.19^{cd}	0.16^{d}	0.030	0.0007
TAC (ng/mL)	0.31^{c}	0.21^{d}	0.56^{bc}	0.73^{ab}	0.81^{a}	0.79^{a}	0.046	0.0001
GSH (mg/dL)	0.38^{bc}	0.22^{c}	0.46^{ab}	0.58^{ab}	0.61^{a}	0.59^{a}	0.039	0.0092
GPX (U/mL)	0.22^{bc}	0.12^{c}	0.34^{ab}	0.33^{ab}	0.36^{a}	0.42^{a}	0.036	0.0337

Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. SOD: Superoxide Dismutase, CAT: Catalase, MDA: Malondialdehyde, TAC: Total Antioxidant Capacity, GSH: Glutathione, GPX: Glutathione Peroxidase.

Table 10: Effects of ginger and garlic essential oils on quails' caecal microbial counts

Items	T1	T2	T3	T4	T5	T6	SEM	P value
TBC	5.66^{bc}	6.10^{a}	5.32^{cd}	5.23^{d}	5.20^{d}	5.32^{cd}	0.036	<0.0001
TYMC	5.68^{bc}	6.00^{a}	4.71^{cd}	4.60^{d}	4.47^{d}	4.37^{d}	0.047	<0.0001
E. coli	5.88^{bc}	6.20^{a}	4.53^{cd}	4.50^{d}	4.57^{cd}	3.56^{e}	0.049	<0.0001
Lactic acid bacteria	3.50^{bc}	2.90^{c}	3.69^{b}	3.78^{b}	4.01^{a}	4.13^{a}	0.061	0.0003
Salmonella	3.42^{bc}	4.10^{a}	2.45^{cd}	2.46^{cd}	2.10^{d}	2.07^{d}	0.047	<0.0001

Significant difference (P<0.05) based on ANOVA with Tukey's post hoc test. Different superscript letters in the same row indicate statistical differences within rows. TYMC: total yeast and molds count. TBC: Total Bacterial Count, TYMC: Total Yeast and Molds Count

Intestinal morphology: Intestinal villus health, critical for nutrient absorption, was severely damaged by heat stress (T2), showing the shortest villi. Supplementation with EOs, especially at higher doses, resulted in a dose-dependent increase in villus length, width, and calculated absorptive surface area. Longer, wider villi in T5 and T6 suggest significant improvements in intestinal integrity and nutrient absorption capacity. Crypt depth variations were also observed, which may relate to altered rates of epithelial cell renewal (Table 11)**.**

Cytokine & HSP expression: The molecular data show that heat stress (T2) significantly upregulated the expression of pro-inflammatory cytokines (IL-1β, TNF-α) and heat shock proteins (HSP70, HSP90). Supplementation with ginger and garlic EOs, particularly at higher doses, markedly downregulated the expression of these markers **(**Fig. 1)**.**

Intestinal histology (H&E Staining): Fig. 2B (T2) demonstrates significant heat-induced enteropathy. Compared to the normal control (T1, Panel A), the intestinal villi appear markedly shortened, blunted, and irregular. There is evidence of villus fusion and a thinning of the columnar epithelial lining on the mucosal surface. The lamina propria may appear edematous, and the overall architecture is disrupted, indicating impaired barrier function and reduced absorptive surface area—a direct histological correlate to the poor nutrient utilization and growth seen in T2. The photomicrographs show a clear, graded recovery from T3 to T6. In groups T3 and T4, villus height and regularity begin to improve. The most significant restoration is observed in T5 and T6 (Fig. 2E & F), where the villi are notably taller, slender, and more uniform than even the non-stressed control (T1). The epithelial lining is intact and densely packed with columnar cells. The muscularis and submucosal layers also appear more organized.

Table 11: Effects of ginger and garlic essential oils on quails intestinal villi morphometry

Intestinal Measurement	T1	T2	T3	T4	T5	T6
Villus length (µm)	850, 912, 1046	550, 600, 645	943, 1024, 1038	1198, 1243, 1321	1256, 1300, 1448	1421, 1538, 1606
Villus width (µm)	53, 66, 67	35, 40, 45	87, 108, 116	93, 99, 112	93, 115, 159	114, 131, 134
Absorptive surface area (mm^2)	0.045, 0.060, 0.070	0.025, 0.030, 0.035	0.082, 0.110, 0.120	0.111, 0.123, 0.147	0.116, 0.149, 0.230	0.162, 0.201, 0.215
Crypt depth (µm)	102, 135, 122	160, 175, 190	146, 186, 196	118, 161, 163	138, 147, 211	201, 204, 208

Liver histology (H&E staining): The liver section from the heat-stressed group (T2) exhibits clear signs of metabolic stress and early injury. While the overall hepatic architecture is maintained, there is likely an increase in cytoplasmic vacuolation (micro- and macro-vesicular steatosis), indicating lipid accumulation or hydropicdegeneration. Sinusoids may appear congested, and there might be signs of inflammatory infiltrate. With EO supplementation, the hepatic histology shows marked improvement. In treated groups, particularly T5 and T6, hepatocytes display minimal vacuolation, dense eosinophilic cytoplasm, and distinct, centrally located nuclei. The hepatic cords are well-organized, and the sinusoids are clear. The portal triads and central veins appear normal (Fig. 3).

Spleen histology (H&E Staining): The spleen in T2 shows characteristic signs of stress-induced lymphoid depletion. The white pulp, composed of periarteriolar lymphoid sheaths and follicles (sites of T- and B-cell activity, respectively), appears reduced in size and cellular density. The lymphoid follicles are likely smaller with less distinct boundaries. The red pulp may show signs of congestion or reduced cellularity. This immune tissue atrophy is structural. Basis for the significantly lower relative weight of the spleen and other immune organs recorded in T2. The progression from T3 to T6 depicts a clear immunostimulatory effect. The white pulp areas become progressively larger and more prominent. In T5 and T6, the lymphoid follicles are substantially enlarged, with clear germinal centers indicating active lymphocyte proliferation. The red pulp remains normally cellular (Fig. 4).

DISCUSSION

The current study offers solid evidence that dietary supplementation with ginger and garlic essential oils (EOs) provides significant, dose-dependent benefits for broiler chickens, especially under heat stress conditions. The results cover a broad spectrum of physiological, immunological, biochemical, and histological parameters, highlighting the diverse roles of these phytogenic additives in poultry production.

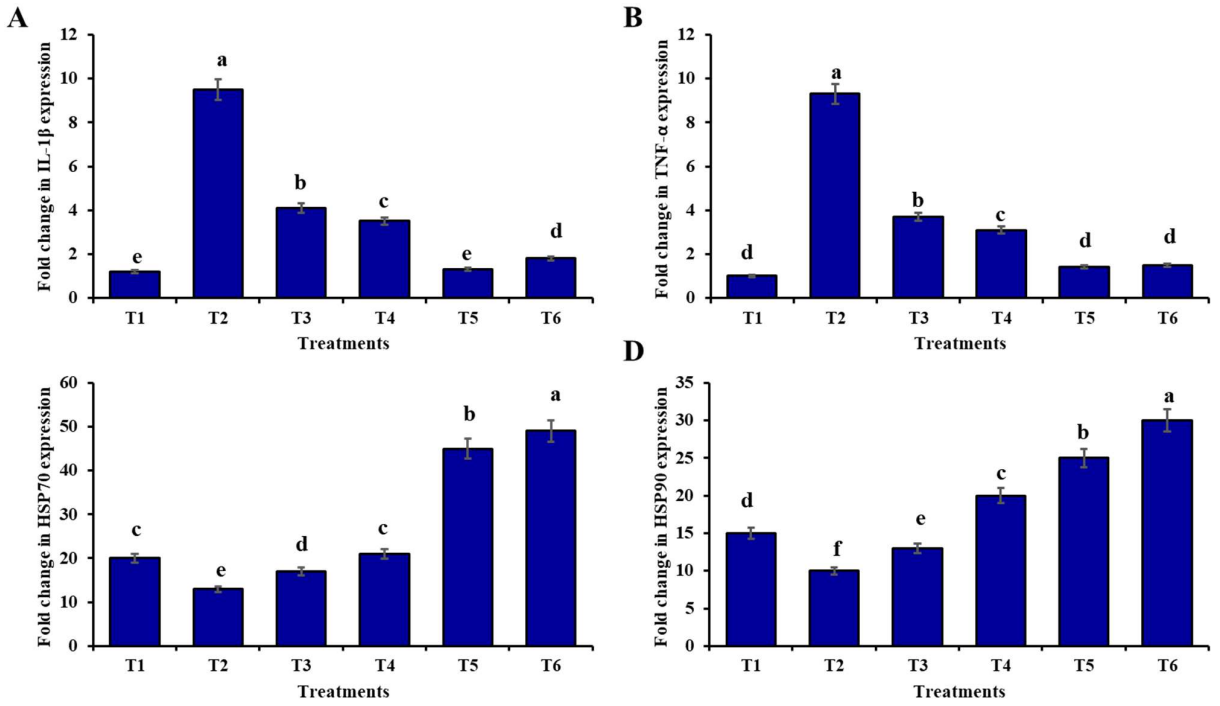

Fig. 1: Effect of Ginger and Garlic Essential Oils on the Expression of Pro-inflammatory Cytokines (A, B) (IL-1β, TNF-α) and Heat Shock Proteins (C, D) (HSP70, HSP90) in Heat-Stressed Experimental Groups. IL-1β: Interleukin 1 Beta, TNF-α: Tumor Necrosis Factor Alpha, HSP70: Heat Shock Protein 70, HSP90: Heat Shock Protein 90. Lowercase letters above the columns indicate significant differences (P<0.05)

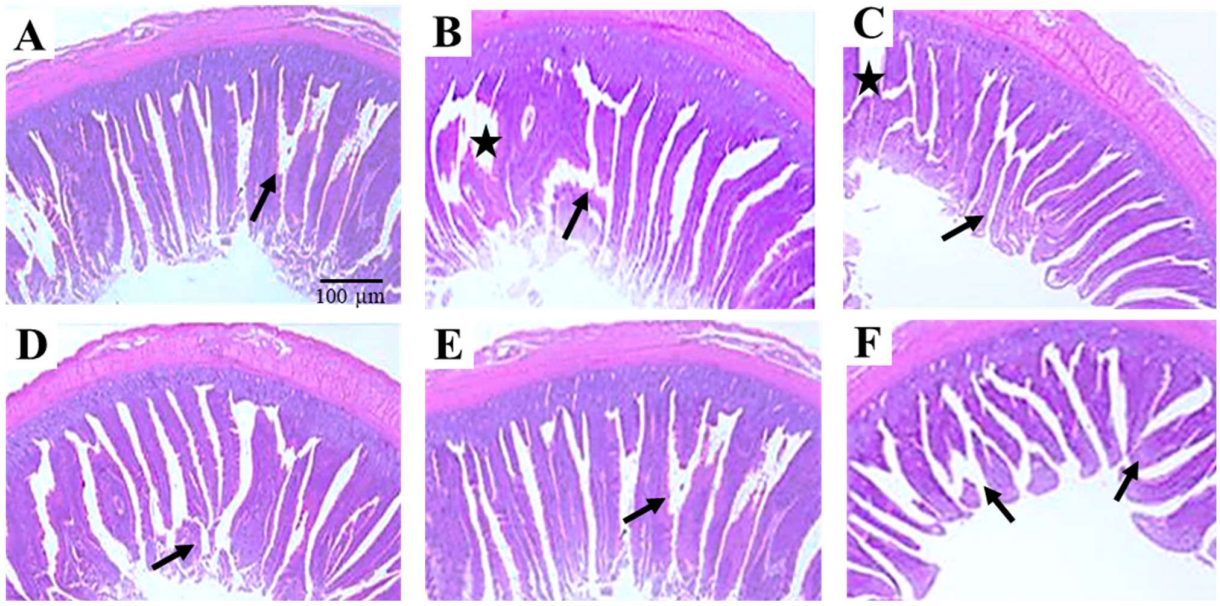

Fig. 2: Photomicrograph sections of intestine stained with H&E (A-F) (scale bar 200µm) showing the histological architectures of the muscular layer, submucosal layer, and columnar epithelial lining of the mucosal villi, with gradually improved integrity of intestinal layers, primarily the mucosal villi, from group 1 to group 6, respectively. (A-F).

The data unequivocally indicate that supplementation with ginger and garlic essential oils significantly enhances growth performance indicators, particularly at elevated dosages (T4, T5, T6). Although initial body weight (BW) and body weight gain (BWG) during the initial fortnight exhibited no notable differences among the experimental groups, by days 28 and 42, the groups receiving essential oil supplementation attained markedly higher final BW and BWG in comparison to both the control group (T1) and the heat-stressed control group (T2). This observation aligns with prior research demonstrating that phytogenic feed additives can improve growth performance in broilers by augmenting nutrient digestibility, stimulating the secretion of digestive enzymes, and modulating gut microbiota (Greathead, 2003; Windisch *et al.,* 2008; Alagawany *et al.,* 2015; Zeng *et al.,* 2015)

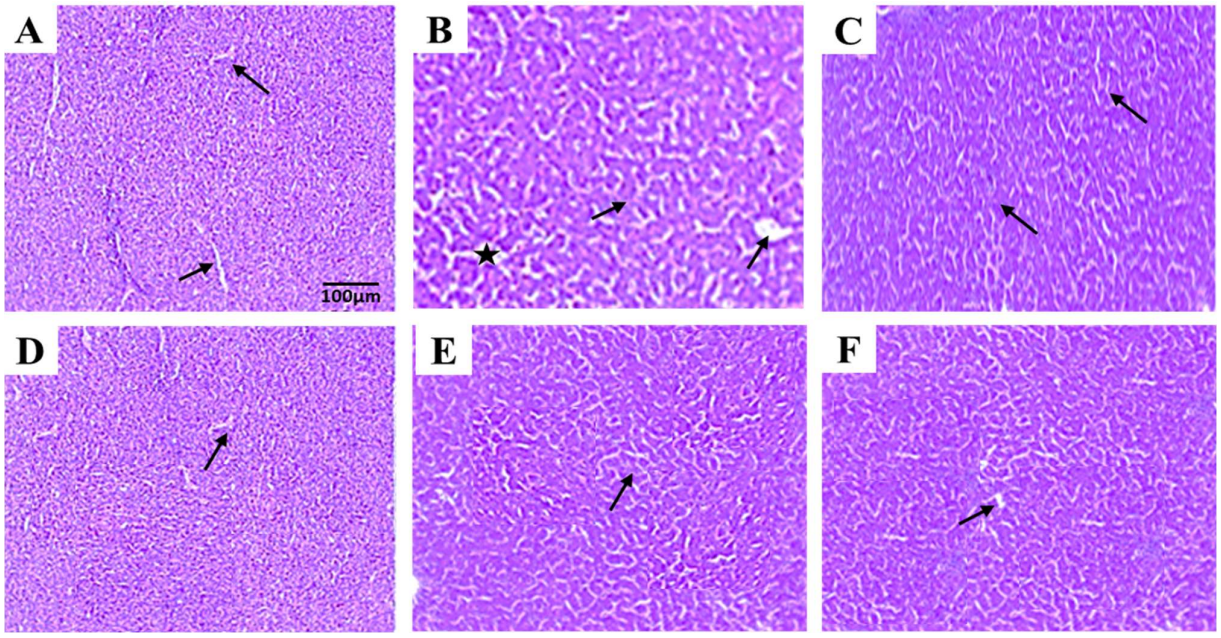

Fig. 3: Photomicrograph of H&E-stained sections of liver (A-F) (scale bar 100µm) showing: Normal morphology of hepatic cells, Kupffer cells, sinusoids, portal triads, and central veins with intracytoplasmic vacuolations with centrally located nuclei, particularly at group 1 (A).

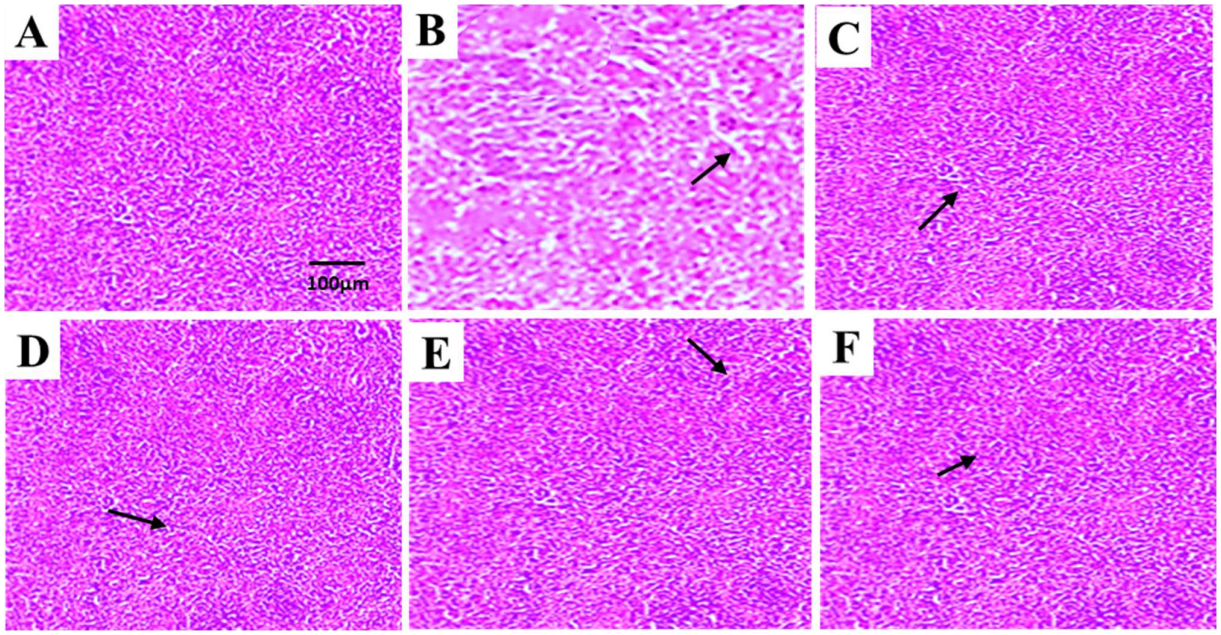

Fig. 4: Photomicrographs of H&E-stained sections of spleen (A-F) (Scale bar 100µm) showing normal histological architectures of white pulp lymphoid follicles and normal red pulp. Gradually increase the diameters of lymphoid masses at white pulps from T1-T6 (A-F), respectively.

Interestingly, feed intake (FI) during the later stages (28-42 and 1-42 days) was significantly lower in EO-treated groups, suggesting that these additives may improve feed efficiency. The combination of higher weight gain and lower feed intake resulted in a substantial and significant improvement in feed conversion ratio (FCR) across the growing period for all supplemented groups. Improved FCR is a critical economic and environmental goal in poultry production, as it indicates more efficient conversion of feed into body mass (Amad *et al.,* 2011).

Carcass characteristics were also positively influenced by EO supplementation. Heat stress (T2) significantly reduced carcass and dressing percentages, but all EO-treated groups recovered these values to or above the level of the non-stressed control (T1), with the highest doses (T5, T6) yielding the most significant improvements. This is in line with studies showing that phytogenic additives can mitigate the negative effects of heat stress on carcass yield and organ development (El-Deep *et al.,* 2016; Liu *et al.,* 2019). The relative weights of liver, gizzard, and heart—collectively referred to as giblets—were depressed by heat stress but were restored or increased with EO supplementation. This suggests that EOs may protect against heat-induced organ atrophy, possibly by reducing oxidative stress and inflammation (Lee *et al.,* 2003; Hashemi & Davoodi, 2012).

The immune system is especially sensitive to heat stress, as shown by the noticeable decrease in the relative weights of key immune organs (spleen, thymus, bursa of Fabricius) in the heat-stressed group (T2). Supplementing with ginger and garlic EOs, particularly at higher doses (1.5 and 2.0mL/kg), significantly increased the relative size of these organs compared to both control groups. The weights of the bursa, thymus, and spleen in T5 and T6 were notably larger, indicating improved lymphoid tissue growth and function. These results are supported by earlier studies that highlight the immunostimulatory effects of ginger and garlic EOs, which are linked to their bioactive compounds such as allicin and gingerol (Toghyani *et al.,* 2011; Hashemi & Davoodi, 2012; Lee *et al.,* 2014).

Heat stress negatively impacted blood parameters, reducing hemoglobin, red blood cell (RBC) count, and white blood cell (WBC) count, while increasing blood glucose levels. EO supplementation reversed these effects, with treated groups, especially T5 and T6, showing significantly higher levels of hemoglobin, RBCs, and WBCs. This indicates improved oxygen transport and immune cell proliferation, which are crucial for maintaining health and performance under stress (Ademola *et al.,* 2009; Seven *et al.,* 2010; Khan *et al.,* 2012). The decrease in blood glucose levels in higher-dose groups suggests better glycemic control or reduced metabolic stress, likely due to the hypoglycemic properties of ginger and garlic (Srinivasan, 2005; Ademola *et al.,* 2009).

Serum biochemistry showed that EO supplementation enhanced protein metabolism and liver health while decreasing kidney workload. Total protein (TP), albumin (ALB), and globulin (GLOB) levels, which were lower in T2, increased significantly in the treated groups. Liver enzyme activities (ALT, AST), indicators of hepatic stress or damage, were notably elevated in T2 but decreased to the lowest levels in EO groups (T5, T6). Similarly, kidney stress markers (creatinine, uric acid) were highest in T2 and significantly reduced by EO treatment. These findings support the hepatoprotective and renoprotective effects of ginger and garlic EOs, likely due to their antioxidant and anti-inflammatory properties (Banerjee *et al.,* 2003; El-Deep *et al.,* 2016).

The lipid profile improved significantly with EO supplementation. Heat stress (T2) was linked to increased levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL). All supplemented groups experienced a notable, dose-dependent decrease in these atherogenic lipids. In contrast, high-density lipoprotein (HDL), known as the "good" cholesterol, significantly rose in the higher-dose groups (T5, T6). These results align with studies indicating the hypolipidemic effects of ginger and garlic, which may stem from their impact on lipid metabolism and antioxidant properties (Ademola *et al.,* 2009; Elagib *et al.,* 2013; Alagawany *et al.,* 2015).

The levels of immunoglobulins (IgM, IgA, IgG) were significantly suppressed by heat stress (T2). Ginger and garlic EO supplementation, particularly at 1.5 and 2.0 ml/kg (T5, T6), significantly boosted the concentrations of these key antibodies. The increase in IgM, IgA, and IgG indicates enhanced systemic and mucosal humoral immune response, which is critical for disease resistance and overall health (Toghyani *et al.,* 2011; Hashemi & Davoodi, 2012; Lee *et al.,* 2014; Abd El-Ghany *et al.,* 2024).

EO supplementation markedly enhanced the antioxidant defense system. Enzymatic antioxidants (SOD, CAT, GPX) and non-enzymatic components (GSH, TAC) were notably elevated in the treated groups in comparison to both the control and heat-stressed groups. Simultaneously, malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative damage, was at its highest in T2 and showed significant reduction in EO groups. This evidence supports the well-recognized antioxidant properties of ginger and garlic constituents, which serve to protect tissues from oxidative injury (Banerjee *et al.,* 2003; Seven *et al.,* 2010; Khan *et al.,* 2012; Zeng *et al.,* 2015).

The synergistic effect of ginger and garlic essential oils (GGEO) operates through a multi-tiered molecular defense system that directly addresses the physiological failures observed in the heat-stressed control (T2). HSP Pathway and Protein Proteostasis: The significant up-regulation of HSP70 and HSP90 mRNA in T5 and T6 serves as a primary defense against the thermal denaturation of cellular proteins. This molecular shielding is reflected in the preserved histological architecture of the liver and spleen and the significantly improved villus length and width, which are typically compromised by protein degradation during heat stress (Surai & Kochish, 2019). Nrf2 Pathway and Redox Balance: The GGEO blend directly countered the oxidative crisis seen in T2, where MDA was highest and TAC, SOD, and GSH were lowest. By increasing TAC and antioxidant enzyme activities in a dose-dependent manner, GGEO inclusion (1.5–2.0mL/kg) likely activated the Nrf2 signaling pathway, inducing the synthesis of endogenous antioxidants that neutralized lipid peroxidation and protected cell membranes (Bogolyubova *et al.,* 2022). NF-κB Inhibition and Anti-inflammatory Response: Heat stress in T2 triggered a massive surge in the pro-inflammatory cytokines IL-1β and TNF-α. The suppression of these genes in T5 and T6 suggests that GGEO components (like allicin and gingerols) inhibit the NF-κB pathway. This reduction in systemic inflammation prevented the leakage of hepatic and renal enzymes, explaining the significantly lower levels of ALT, AST, and Creatinine in supplemented groups (Asdaq *et al.,* 2022). Nutrient Partitioning and Performance: By stabilizing these pathways, GGEO reduced the metabolic cost of the stress response (evidenced by lower glucose levels in T4–T6). This allowed for the partitioning of nutrients toward growth rather than immune maintenance, resulting in the optimized FCR (1.73) and superior final body weight observed in the high-inclusion groups (Hosseindoust *et al.,* 2022).

EO supplementation exerted significant modulatory effects on caecal microflora. The heat-stressed group (T2) exhibited the highest counts of total bacteria (TBC), yeast/mold (TYMC), *E. coli*, and *Salmonella*. Supplementation markedly reduced these potentially harmful microbes in a dose-dependent manner. Conversely, the population of beneficial lactic acid bacteria increased substantially in groups T5 and T6. This indicates a prebiotic effect, supporting gut health and competitive exclusion of pathogens (Lee *et al.,* 2003; Windisch *et al.,* 2008; Hashemi & Davoodi, 2012).

Intestinal villus health, critical for nutrient absorption, was severely damaged by heat stress (T2), showing the shortest villi. Supplementation with EOs, especially at higher doses, resulted in a dose-dependent increase in villus length,

width, and calculated absorptive surface area. Longer, wider villi in T5 and T6 suggest significant improvements in intestinal integrity and nutrient absorption capacity. Crypt depth variations were also observed, which may relate to altered rates of epithelial cell renewal. These morphological benefits are in line with studies showing that phytogenic additives promote intestinal health and nutrient absorption (Amad *et al.,* 2011; Zeng *et al.,* 2015; Liu *et al.,* 2019).

The molecular data show that heat stress (T2) significantly upregulated the expression of pro-inflammatory cytokines (IL-1β, TNF-α) and heat shock proteins (HSP70, HSP90). Supplementation with ginger and garlic EOs, particularly at higher doses, markedly downregulated the expression of these markers, indicating anti-inflammatory and cytoprotective effects (El-Deep *et al.,* 2016). Histological analyses further confirm the protective and restorative effects of EOs on intestinal, hepatic, and splenic tissues under heat stress. In the intestine, EOs supplementation led to taller, more regular villi and restored epithelial integrity. In the liver, EOs-treated groups showed minimal vacuolation and well-organized hepatic cords, while in the spleen, lymphoid follicles were enlarged and more cellular, indicating active immune function (Lee *et al.,* 2010).

While the present study provides robust evidence for the protective effects of a ginger and garlic essential oil (GGEO) blend against heat stress, several limitations should be acknowledged. First, this research was conducted using a single broiler strain (Arbor Acre) in a controlled experimental setting. Consequently, the physiological responses observed may vary across different genetic lines or in large-scale commercial poultry operations where environmental variables—such as ammonia levels, stocking density, and fluctuating humidity—are less regulated. Furthermore, while we identified 2.0mL/kg as the most effective dose among the tested levels, the study did not employ advanced dose-response modeling (e.g., broken-line or non-linear regression) to pinpoint the precise biological optimum or the threshold for potential phytogenic toxicity.

Future research should validate these findings across diverse climatic conditions and broiler breeds to ensure broader applicability. Additionally, investigating the economic feasibility of long-term GGEO supplementation in commercial diets is essential. Further molecular studies are also warranted to explore the interplay between GGEO and other metabolic pathways, such as the Nrf2/ARE signaling cascade at the protein level, to fully elucidate the nutrigenomic potential of these essential oils in mitigating environmental stressors.

Conclusions: The results of this study provide robust evidence that ginger and garlic essential oils can effectively mitigate the detrimental effects of heat stress in broiler chickens. The benefits observed include improved growth performance, feed efficiency, carcass traits, immune organ development, hematological and biochemical parameters, antioxidant status, gut health, and tissue integrity. These findings support the use of ginger and garlic EOs as natural growth promoters and health enhancers in poultry production, offering a promising alternative to synthetic additives and antibiotics.

Authors contribution: Conceptualization**,** AA, NMF, RO, SMAB, FAA, MAG, and AMAO, formal analysis, MAA, AAA, SAA, MAM, AEA, and MTES, investigation, AA, NMF, RO, SMAB, FAA, MAG, and AMAO, data curation, MAA, AAA, SAA, MAM, AEA, and MTES, writing original draft preparation, AA, NMF, RO, SMAB, FAA, MAG, and AMAO, writing final manuscript and editing, MAA, AAA, SAA, MAM, AEA, and MTES, visualization and methodology, AA, NMF, RO, SMAB, FAA, MAG, AMAO, MAA, AAA, SAA, MAM, AEA, and MTES. All authors have read and agreed to the published version of the manuscript.

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Ethical approval for the study: The animal experiment was conducted at Zagazig University, Zagazig, Egypt. The study protocol received review and approval from the Institutional Animal Care and Use Committee (IACUC). All methods were implemented to reduce animal suffering and use the minimum number of animals necessary to obtain reliable scientific data.

Declaration of competing interest: The authors declare that they have no conflict of interest.

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