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Fennel essential oil as a natural feed additive to mitigate heat stress in broilers: Effects on growth, physiological and immunological responses, and intestinal morphology

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ABSTRACT

This study examined how dietary fennel essential oil (FEO) supplementation affected heat-stressed (HS) broiler chickens' growth, economic efficiency, hematological and biochemical blood profiles, immunological response, antioxidant status, and intestinal histomorphology. A total of 600 one-day (d)-old Ross 308 chicks were assigned to 6 treatments: thermoneutral control (TNC), HS control (HSC), three HS groups with 1, 2, or 3 g FEO/kg diet, and a HS group given paracetamol (500 mg/L drinking water). Birds were raised under TN settings until d 21, exposed to 36 ± 0.5 °C for 6 h/d from d 22–25, and then returned to TN conditions until d 35. Dietary FEO supplementation had no effect on growth during the starter phase ($P > 0.05$). The grower phase showed significant treatment effects on growth performance indices ($P < 0.05$), with FEO- and paracetamol-supplemented birds exhibiting comparable body weight (BW) and body weight gain (BWG) to HSC. FI was reduced in FEO-fed groups relative to both HSC and paracetamol group ($P < 0.001$), while FCR remained comparable among these groups. Finisher phase and overall performance showed enhanced BW, BWG, and FCR in broilers fed 1–3 g FEO/kg versus HSC, despite comparable FI among these groups ($P < 0.001$). FEO supplementation increased red blood cell count, hemoglobin concentration, packed cell volume, lymphocyte count, and enhanced key biochemical, immunological, and antioxidant markers ($P < 0.05$). Improved small intestinal histomorphology, increased splenic $CD3^+$ T-cell and $CD20^+$ B-cell immunostaining, enhanced breast muscle fatty acid profile, and greater economic efficiency were also observed in FEO-supplemented birds ($P < 0.05$). Overall, dietary FEO supplementation improved the resilience of broilers exposed to HS, suggesting it may serve as a viable nutritional strategy for mitigating performance losses associated with HS.

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1. Introduction

The poultry production industry is a significant sector within the global agricultural industry. The demand for poultry for human consumption has increased as the world's population grows, and since 1990, the yearly increase in chicken meat has averaged 5.7% (Delgado, 2005). In spite of its growth, poultry production is exceedingly susceptible to environmental stressors, especially heat stress (HS), which acts as a substantial obstacle to worldwide poultry production (Apalowo et al., 2024; Thieme and Pilling, 2008; Wasti et al., 2020). This challenge is evident in subtropical countries such as Egypt, where summer season is characterized by high ambient temperatures (35–40 °C), high relative humidity (50–75%), and intense solar radiation (Habeeb, 2020). Extremes of these conditions usually occur during periods of maximum HS, which last over six months, from May to October (Habeeb, 2020). These seasonal conditions cause significant animal stress, negatively impacting their growth performance and health status (Apalowo et al., 2024; Gouda et al., 2024; Habeeb et al., 2018).

Heat stress arises when the balance between heat loss and production is disrupted. Because poultry lack sweat glands, they dissipate excess sweat under high ambient temperature via panting, potentially resulting in significant physiological imbalances that disrupt poultry growth performance, immunological function, and overall health (Apalowo et al., 2024; Gouda et al., 2024). Significantly, HS can promote oxidative stress by disturbing the balance between the production of free radicals and antioxidant defense systems, leading to cellular damage to lipids, proteins, and nucleic acids (Gouda et al., 2024). Antioxidants, which are stable compounds capable of donating electrons to free radicals, are critical in diminishing such damage. Inside the body, various free radicals are neutralized by various enzymatic systems alongside a non-enzymatic component, such as micronutrients, to neutralize free radicals (Oke et al., 2024). Phytochemicals are a particular type of nutrient that helps protect the oxidative system under stressful conditions (Farag, 2025; Mahasneh et al., 2024). According to earlier research, adding phytochemicals to the diet may enhance chicken feed intake, improve performance indicators, and modify the quality of poultry products (Insawake et al., 2025; Jimoh et al., 2022). Such interventions have gained increasing relevance since the European Union's prohibition on using antibiotics as feed additives in chicken diets in 2006, following the concerns over the emergence of antibiotic-resistant bacteria and associated risks to human health and animal production (Ungemach et al., 2006).

Fennel (*Foeniculum vulgare*) is a recognized herbal plant that traditionally used to treat gastrointestinal and respiratory disorders (Gende et al., 2009; Noreen et al., 2023; Rather et al., 2016). Under thermoneutral conditions, fennel has demonstrated the capacity to promote weight gain, enhance egg quality, and improve digestion in poultry at levels of 0.5 to 1% (El-Deek et al., 2003), 10 to 20 mg/kg (Gharaghani et al., 2015), and 1.6 to 3.2 mg/kg (Al-Sagan et al., 2020). The benefits of fennel are attributed to the fennel's rich phytochemical composition, which includes volatile components, flavonoids, hydrocarbons, phenolic compounds, fatty acids, and amino acids (Badgujar et al., 2014). Earlier research has determined trans-anethole as a primary volatile component of fennel seed essential oil (FEO), which predominantly contributes to its antibacterial effectiveness in organisms (Ghasemian et al., 2020; Shahat et al., 2011). Yu et al. (2021) reported that incorporating varying levels of trans-ethanol into diets elevated the average daily feed intake (FI) of broiler chickens, but did not influence average body weight gain (BWG), feed-to-gain ratio, or body weight (BW) throughout the experimental duration. In contrast, 400 mg/kg of trans-ethanol increased crypt depth (CD), villus height (VH), the VH: CD ratio, and *Bifidobacterium* populations, while decreasing *Escherichia coli* populations (İpçak et al., 2024). Furthermore, the literature indicates that incorporating fennel extract into the diets of laying hens may mitigate the adverse effects of carbon tetrachloride (Hadavi et al., 2017). This hepatic toxin elevates the production of reactive oxygen species (ROS) by neutralizing oxidants in the damaged liver, thereby enhancing liver health and egg production (Hadavi et al., 2017).

Limited information is available regarding the efficacy of FEO supplementation in poultry in the context of HS. To our knowledge, fennel seed powder has been the primary focus of previous research rather than its essential oil. For instance, an earlier study showed that dietary inclusion of fennel seed powder at doses of 20 and 25 g/kg enhanced the immune response, growth performance, carcass characteristics, and antioxidant status of broilers raised under HS conditions (Fatima et al., 2022). Administration of fennel extract to laying hens under HS reduced levels of malondialdehyde and protein carbonyl in their eggs, alleviating oxidative damage and diminishing the adverse effects of free radicals (Gharaghani et al., 2015). Importantly, in addition to growth performance and other health status parmaeters, evaluating the economic efficiency of dietary FEO supplementation is crucial to ascertain its practical feasibility, as enhancements in performance must be weighed against feed cost and overall production profitability in commercial poultry production systems (Hamouda et al., 2025; Zuidhof et al., 2014). However, data regarding the economic effeciency of supplementing FEO into broiler diets under HS conditions is limited.

Therefore, this study was designed to evaluate the potential impact of different levels of dietary FEO supplementation on growth performance, economic efficiency, antioxidant capacity, metabolic profile, immune response, and intestinal histomorphology of broiler chickens exposed to acute HS conditions. We hypothesized that dietary FEO supplementation would alleviate HS-induced physiological and oxidative disruptions, therefore enhancing broiler performance and economic efficiency.

2. Materials and methods

2.1. Gas Chromatography–Mass spectrometry (GC-MS) analysis of FEO

Fennel essential oil was obtained from Imtenan for Natural Products (Cairo, Egypt). The active compounds of FEO were determined using a Trace GC1310-ISQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA), with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 μm film thickness), following the previous description of Amer et al. (2022a). The bioactive compounds of FEO are shown in Table 1.

2.2. Birds and experimental design

A total of 600 one-day (d)-old male Ross 308 broiler chicks were obtained from a commercial hatchery and brooded under standard management conditions in accordance with Aviagen guidelines (Aviagen, 2025). At 3d of age, the chicks were individually weighed (initial weight 93.39 ± 3.37 g) and randomly allocated to six experimental treatment groups in a completely randomized design (10 replicates per group, 10 birds per replicate). Each pen (160 × 240 cm) had

Table 1
Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the fennel essential oil.

Bioactive Compounds	Retention Time	Peak Area %
Estragole	20.16	16.57
Anethole	22.86	17.44
9-Octadecenoic acid (Z)-, methyl ester	51.83	8.36
D-Limonene	11.72	13.41
(−)-Carvone	20.83	3.90
Hexadecanoic acid, methyl ester	46.56	5.19
Octadecanoic acid, methyl ester	52.69	1.92
9,12-Octadecadienoic acid (Z, Z)-, methyl ester	51.50	3.71
Fenchone	13.32	1.06

wood shavings as bedding and was equipped with circular feeders and drinkers. The experimental treatments were as follows: a thermoneutral control group receiving a control basal diet (TNC), a heat-stressed control group receiving the control basal diet (HSC), three heat-stressed groups supplemented with FEO at 1 g/kg (FEO1), 2 g/kg (FEO2), and 3 g/kg (FEO3) of diet, and a heat-stressed group receiving paracetamol (500 mg/L) in drinking water. According to the commercial temperature management guidelines (Aviagen, 2025), all birds were kept in TN conditions from d 1 to 21; 32 °C during the first week, reduced to 28 °C on d 7, 26 °C on d 14, and then decreased by 1 °C every two days until reaching 23 °C on d 21. From d 22 to 25, HS was induced in the designated groups by increasing the ambient room temperature by approximately 13 °C above the recommended level, reaching 36 ± 0.5 °C for 6 h per day. From d 26 until the end of the experiment (d 35), all birds were returned to TN conditions following the standard temperature schedule. As per the standard protocol of Ross 308 broiler nutrition requirements (Aviagen, 2022), the birds were fed a corn-soybean meal-based starter diet until they were 10 d old. After that, they were fed a grower diet from d 11-23 and a finisher diet from d 24-35 (Table 2). Fresh water and mash feed were given *ad libitum* during the experiment. A standard vaccination program against Newcastle disease in addition to other common infectious diseases was applied uniformly to all birds following the recommendations of Aviagen (2025).

2.3. Growth performance

The average BW was determined on the third day of age to determine the initial BW. Subsequently, birds were weighed on d 10, 23, and 35 to determine the average BW at various rearing stages (starter, grower, and finisher); thereafter, BWG was computed. The difference between the weight of the supplied feed and the leftover feed has been recorded to determine FI per replicate. The feed conversion ratio (FCR) was then calculated as: FCR = FI(g) / BWG(g) (Amer et al., 2022b).

2.4. Sample collection

Ten birds from each treatment were randomly selected for sample collection at the end of the study (d 35). For hematological analysis, whole blood samples (~1 ml) were collected from live birds via wing vein puncture, using heparinized syringes. The samples were immediately transferred into heparinized tubes, promptly preserved on ice in a chilled box, and transported to the laboratory for analysis within 2 h of collection. Following whole blood sampling, birds were euthanized by cervical dislocation in accordance with the American Veterinary Medical Association guidelines (Association, 2013). For biochemical analysis, blood samples were subsequently collected in vacutainer tubes devoid of anticoagulant (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). The tubes were left to coagulate at ambient temperature and centrifuged at 3000×*g* for 13 min to obtain serum, which was stored at – 20 °C until further analysis.

Liver sections were immediately snap-frozen in liquid nitrogen and stored at – 80 °C for further molecular analysis. Around 2 cm sections from various segments of the small intestine (duodenum, jejunum, and ileum) and from the spleen were collected and placed in 10% neutral buffered formalin for further histomorphological and immunohistochemical analysis (Giannenas et al., 2010); Sikandar et al. (2017).

2.5. Fatty acid composition of breast muscle

Ten breast muscle samples (~50 g each) were collected from each group for fatty acid analysis. According to Belitz et al. (2009), a chloroform/methanol (2:1, v/v) solvent technique was employed for fat extraction. The extracted fatty acids were subsequently measured in accordance with AOAC (2000).

2.6. Hematological analysis

According to the manufacturer's instructions, a Hemascreen 18 Automatic Cell Counter (Hospitex Diagnostics, Italy) was used to

Table 2
Ingredients and composition (g/kg) of the basal diet.

Ingredients (%)	Control diet[a]			FEO 1 g/kg diet			FEO 2 g/kg diet			FEO 3 g/kg diet		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
Yellow corn	557.25	592.5	622	557.25	592.5	622	557.25	592.5	622	557.25	592.5	622
Soybean meal, 48%	335.3	280	236	335.3	280	236	335.3	280	236	335.3	280	236
Corn gluten, 60%	40	53.25	60	40	53.25	60	40	53.25	60	40	53.25	60
FEO	0	0	0	1	1	1	2	2	2	3	3	3
Soybean oil	22	31	40.95	21	30	39.95	20	29	38.95	19	28	37.95
Calcium carbonate	12	12	11	12	12	11	12	12	11	12	12	11
Dicalcium phosphate 18%	15	14	13	15	14	13	15	14	13	15	14	13
Nacl	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Premix[2]	3	3	3	3	3	3	3	3	3	3	3	3
DL- Methionine, 98%	4	3	3.3	4	3	3.3	4	3	3.3	4	3	3.3
Lysine HCl, 78%	4.7	4.5	4	4.7	4.5	4	4.7	4.5	4	4.7	4.5	4
Choline	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
L-Threonine 98.5%	1	1	1	1	1	1	1	1	1	1	1	1
Phytase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium bicarbonate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Antimycotoxin	1	1	1	1	1	1	1	1	1	1	1	1
Chemical composition (g/kg)												
ME (kcal/kg)	3003	3107	3208	3003	3107	3208	3003	3107	3208	3003	3107	3208
CP	231.2	215	200.2	231.2	215	200.2	231.2	215	200.2	231.2	215	200.2
Crude fat	49.6	58.9	69.1	49.6	58.9	69.1	49.6	58.9	69.1	49.6	58.9	69.1
Crude starch	406.2	429.7	449	406.2	429.7	449	406.2	429.7	449	406.2	429.7	449
Lysine	14.7	13.1	11.6	14.7	13.1	11.6	14.7	13.1	11.6	14.7	13.1	11.6
Methionine	7.2	6.1	6.3	7.2	6.1	6.3	7.2	6.1	6.3	7.2	6.1	6.3
Calcium	9.4	9	8.3	9.4	9	8.3	9.4	9	8.3	9.4	9	8.3
Av. P	4.8	4.5	4.2	4.8	4.5	4.2	4.8	4.5	4.2	4.8	4.5	4.2

[2.4.] [2]Premix per kg of diet: vitamin D3, 200 IU; vitamin A, 1500 IU; vitamin K3, 0.5 mg; vitamin E, 10 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; folic acid, 0.55 mg; pantothenic acid, 10 mg; niacin, 35 mg; pyridoxine, 3.5 mg; biotin, 0.15 mg; cobalamin, 0.01 mg; Zn, 40 mg; Fe, 80 mg; Mn, 60 mg; Cu, 8 mg; Se, 0.15 mg I, 0.35 mg. FEO: fennel essential oil, ME: metabolizable energy, CP: crude protein, Av. P: available phosphorus.*Sample collection.*

[a] The control diet was fed to the thermoneutral and heat-stressed control groups.

determine differential leucocytic counts, total red blood cells (RBCs), and white blood cells (WBCs) (Yang et al., 2018). The hemoglobin (Hb) concentration was determined using the cyanmethemoglobin method as described by Drabkin and Austin (1932) with a colorimetric reagent (BioDiagnostic, Egypt). To ascertain hematocrit ratios, the packed cell volume (PCV) was measured after centrifugation of blood samples in Wintrobe hematocrit containers at 2000×*g* for 20 min at 4 °C. The following equations were employed to calculate the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC):

$$\text{MCV (fL)} = \text{HT (\%)} / \text{RBC } (10^6\ \mu\text{L}^{-1}) \times 10$$

$$\text{MCH (pg)} = \text{HB (g dL}^{-1}) / \text{RBC } (10^6\ \mu\text{L}^{-1}) \times 10$$

$$\text{MCHC (\%)} = \text{HB (g dL}^{-1}) / \text{HT (\%)} \times 100$$

2.7. Blood biochemical analysis

The methodology constructed by Sánchez-Carbayo et al. (1999) was used to assess blood levels of triiodothyronine (T_3) and thyroxine (T_4) using commercially available kits (Byk-Sangtec Diagnostica, Dietzenbach, Germany) in accordance with the manufacturers' guidelines.

Serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined using the Spectrum-Bioscience colorimetric diagnostic kits (Egyptian Company for Biotechnology, Cairo, Egypt), as previously established by Allain et al. (1974), McGowan et al. (1983), and Vassault et al. (1986), respectively. The low-density lipoprotein cholesterol (LDL-C) level was determined using the Iranian formula LDL-C = TC/1.19 + TG/1.9 – HDL-C/1.1 (AHMADI et al., 2008). The turbidimetric method established by Griffin and Whitehead was employed to quantify very low-density lipoprotein cholesterol (VLDL-C) (Griffin and Whitehead, 1982).

2.8. Immune status assessments

Serum levels of interleukin-10 (IL-10) and complement-3 (C3) were determined with chicken-specific ELISA kits from My BioSource Co. (CAT.NO. MBS701683) and Life Span Biosciences, Inc. (CAT. NO. LS-F9287), respectively. The serum lysozyme activity was measured using the method described by Lie et al. (1986). Phagocytic activity and phagocytic index were assessed following the methods described by Kawahara et al. (1991) and El-Kassas et al. (2018), utilizing *Candida albicans* (*C. albicans*). Briefly, 50 μg of *C. albicans* culture was added to 1 ml of heparinized blood and incubated in a shaking water bath at 25 °C for 5 h. Giemsa-stained blood samples were subsequently prepared. Phagocytic activity was determined as the proportion of phagocytic cells that ingested yeast cells, whilst phagocytic index was evaluated as the average number of yeast cells engulfed per phagocytic cell.

2.9. Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted and purified from ~20 mg of the liver tissue using TRIzol™ reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity and quality of the RNA were assessed using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Complementary DNA (cDNA) was synthesized using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), following the manufacturer's protocol. Quantitative Real-Time PCR (qRT-PCR) (Bio-Rad CFX384 Real-Time PCR Detection System) was used to quantify transcript expression levels of key heat shock proteins 70 (HSP70), 90-α (HSP90-α), and- β (HSP90-β) genes. Primer sequences for the assessed genes are listed in Table 3. Relative gene expression was calculated using the $2^{\wedge}{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference (housekeeping) gene. All values were normalized to the control group and expressed relative to the mean expression level of control birds, which was set to "1".

Table 3
Primer sequences and target genes used for real-time qPCR reactions.

Primer name[a]	Sequence	Accession number
HSP70	F:5″- GATCTGGGCACCACGTATTCT -3″ R:5″- GGTTCATTGCCACTTGGTTCTT -3″	FJ217667.1
HSP 90-α	F:5″- ACA CAT GCC AAC CGC ATT TA -3″ R:5″- CCT CCT CAG CAG CAG TAT CA -3″	NM_001109785.1
HSP 90-β	F:5″- AGT AGA GAA GGG TCC CGA AC -3″ R:5″- GGA TGC ATT TAG GCC ATC CA -3″	NM_204289.1
GAPDH	F:5″- CTTTGGCATTGTGGAGGGTC -3″ R:5″- ACGCTGGGATGATGTTCTGG-3″	NM_204305

[a] Abbreviation: HSP70: heat shock protein70; HSP90-α: heat shock protein90-α; HSP90-β: heat shock protein90-β; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

2.10. Immunohistochemical examination

Spleen tissues were examined for $CD3^+$ T cells and $CD20^+$ B lymphocytes using immunohistochemical analysis following the method described by Saber et al. (2019). The samples were fixed in 10% neutral buffered formalin for four days, then processed using standard histological procedures, including dehydration, embedding in paraffin wax, and microtome sectioning (Bancroft and Gamble, 2008). The paraffin-embedded sections were deparaffinized in xylene and rehydrated in ethyl alcohol. Immunohistochemical staining was performed as previously indicated (Amer et al., 2022b). Slides were initially treated with anti-chicken CD3 (Bio-Rad Lab., Dubai, United Arab Emirates) and CD20 (ThermoFisher Scientific, Waltham, MA, USA). Subsequently, Slides were evaluated as previously described by Amer et al. (2022b). The average grayscale represented the intensity (Lie et al., 1986).

2.11. Histoomorphological examination of the spleen and small intestine

The spleen and small intestine samples were preserved for 72 h in 10% neutral buffered formalin. After fixation, the specimens were cleaned, dehydrated, and embedded in paraffin wax. A Leica RM 2155 microtome (Leica Microsystems, UK) was used to perform histological analysis on transverse slices that were 5 μm thick. After that, the sections were placed on glass slides and stained using hematoxylin and eosin in accordance with Bancroft and Gamble (2008) protocol. While the CD was measured from the villus-crypt junction to the base of the crypt, the VH was measured from the tip of the villus to the villus-crypt junction (Amer et al., 2020). Measurements were conducted using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.12. Economic efficiency

Collective efficiency metrics were calculated to evaluate the economic implications of the experimental treatments using established methodologies described by Dunning (2001) and El-Telbany and Attallah (2000a). These metrics give a thorough picture of economic performance and include feed cost, total cost, total return, net profit, feed cost per kilogram (kg) of BWG, economic efficiency. Furthermore, the performance index was calculated according to the methodology described by North and Bell (1984). The economic indices were calculated using the following equations:

Total feed cost (USD/bird) = Total FI per bird × price of 1 kg of feed.

Total cost (USD/bird) was calculated by accounting for feed expenses together with the costs of 1-d-old-chicks, litter, labor, veterinary services, electricity, and other miscellaneous expenditures that were common to all experimental groups.

Total return (USD/bird) = Live BW per bird × price per kg of live BW.

Net profit (USD/bird) = Total revenue – Total expenses.

Economic efficiency = Net profit/Total feed cost.

Feed cost per kg of BWG (USD/kg BWG) = Total feed cost/total BWG.

Performance index (%) = [Final live BW (kg)/FCR] × 100.

2.13. Statistical analysis

All data were subjected to one-way analysis of variance using SPSS version 28.0 for macOS (SPSS, Inc., Chicago, IL, USA). The mathematical and statistical model used was: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the observation for the dependent variables from each experimental unit; μ = the overall mean; T_i = the treatment effect; εij = random error components of the study. The replicate served as the experimental unit for growth performance assessments, while individual blood draws and health status represented the experimental unit for other parameters. Prior to analysis, The Shapiro–Wilk test was employed to assess normality, whereas Levene's test was utilized to evaluate the homogeneity of variance among experimental treatments. The significant differences between mean values were examined using Tukey's honest significant difference test. Pooled standard errors (SEM) were determined for all analyses, with a significant level established at $P < 0.05$.

3. Results

3.1. Effects of dietary FEO supplementation on growth performance

Table 4 summarizes the effects of dietary FEO supplementation on broiler growth performance. Initial BW and growth performance metrics during the starter phase (4–10 d) did not show significant changes (P = 0.332–0.896) across treatments. During the grower phase (11-23 d), significant treatment effects were observed in growth performance indices ($P < 0.05$), with FEO- and paracetamol-supplemented birds were comparable to the HSC in BW and BWG. FI was reduced in all FEO-fed groups by approximately 1.2–1.7% relative to the HSC and by 2.3–2.8% compared with the paracetamol group ($P < 0.001$), while FCR remained comparable. During the finisher phase (24-35 d), growth performance indices differed among treatment ($P < 0.001$). Relative to HSC, BW increased by 1.9–3.2%, BWG increased by 5.4–6.7%, and FCR was improved by 8.6–10.0% in all FEO- supplemented groups. When compared with the paracetamol-supplemented group, FEO-supplementation increased BW by 0.5–1.8%, increased BWG by 2.0–3.2%, and improved FCR by 4.2–5.6%. FI was comparable between all FEO- and paracetamol-supplemented groups with the HSC. Over the entire experimental period, FEO supplementation enhanced BWG by 2.0–3.4% and improved FCR by 4.6–5.9% relative to the HSC ($P < 0.001$), while FI remained comparable between all FEO-fed groups and the HSC.

3.2. Fatty acid composition of the breast muscle

Table 5 presents the effects of dietary FEO supplementation on the fatty acid composition of breast muscle. Myristic acid (C14:0) differed among treatments (P = 0.040), with higher values observed in the paracetamol-supplemented group than in the HSC, while dietary FEO-supplemented groups remained comparable to the HSC. Linoleic acid (C18:2 n-6) increased in birds fed 1–3 g FEO/kg and in the paracetamol compared with the HSC ($P < 0.001$). Likewise, α-linolenic acid (C18:3 n3) was higher in birds fed 3 g FEO/kg compared with the HSC (P = 0.017). Birds fed 1–3 g FEO/kg had higher polyunsaturated fatty acids (PUFA) contents compared with the HSC ($P < 0.001$).

3.3. Hematological responses

As shown in Table 6, Hb and PCV values were higher in birds supplemented with 1–3 g FEO/kg compared to the HSC (P = 0.006 and P = 0.002, respectively). Moreover, the RBCs count was higher in birds fed 1 and 2 g FEO/kg than in the HSC (P = 0.014). No significant differences were detected among the groups in platelet count, MCV, MCH, and MCHS ($P > 0.05$). For leukogram indices, total WBCs count was

Table 4

Effect of the experimental treatments on the growth performance of broiler chickens.

Parameter	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
Initial BW, g	95.44	93.33	92.11	93.44	92.78	93.22	0.459	0.436
Starter (0–10 d)								
BW, g	297.11	290.78	285.11	287.78	293.78	289.44	1.623	0.332
BWG, g	201.66	197.44	193.00	194.33	201.00	196.22	1.602	0.570
FI, g	292.00	286.67	285.00	287.00	287.00	285.67	0.732	0.082
FCR	1.45	1.46	1.48	1.48	1.43	1.46	0.011	0.896
Grower (11–23 d)								
BW, g	1266.22[a]	1206.78[b]	1223.89[ab]	1204.00[b]	1184.44[b]	1204.44[b]	5.870	<0.001
BWG, g	969.11[a]	916.00[ab]	938.78[ab]	916.22[ab]	890.67[b]	915.00[b]	5.963	0.002
FI, g	1245.00[ab]	1244.67[ab]	1225.00[c]	1229.67b[c]	1223.33[c]	1258.33[a]	2.458	<0.001
FCR	1.28[b]	1.36[a]	1.31[ab]	1.34[ab]	1.38[a]	1.38[a]	0.008	0.002
Finisher (24–35d)								
BW, g	2421.44[a]	2147.22[c]	2215.22[b]	2205.78[b]	2187.67[b]	2176.67[ab]	12.93	<0.001
BWG, g	1155.22[a]	940.44[c]	991.33[b]	1001.78[b]	1003.22[b]	972.22[ab]	10.00	<0.001
FI, g	1975.00[a]	1857.00[ab]	1786.00[b]	1806.11[b]	1782.56[b]	1830.56[b]	15.46	<0.001
FCR	1.71[c]	1.97[a]	1.80b[c]	1.80[bc]	1.78b[c]	1.88[ab]	0.017	<0.001
Overall performance (3–35 d)								
BWG, g	2326.00[a]	2053.89[d]	2123.11[b]	2112.33[bc]	2094.89[bc]	2083.44[cd]	12.81	<0.001
FI, g	3512.00[a]	3388.33[ab]	3296.00[b]	3322.78[b]	3292.89[b]	3374.56[ab]	16.41	<0.001
FCR	1.51[c]	1.65[a]	1.55[bc]	1.57[bc]	1.57[bc]	1.62[ab]	0.009	<0.001

[a,b,c,d] Means with different superscripts within the same row differ significantly ($P < 0.05$). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio.

Table 5
Effect of the experimental treatments on the fatty acid composition (% of total fatty acids) in the breast muscle of broiler chickens.

Parameters	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
C14:0	0.022^{ab}	0.021^{b}	0.022^{ab}	0.021^{ab}	0.022^{ab}	0.023^{a}	0.000	0.040
C16:0	0.857	0.807	0.885	0.840	0.858	0.885	0.009	0.082
C18:0	0.291	0.293	0.290	0.288	0.295	0.294	0.001	0.780
C16:1	0.196	0.194	0.196	0.198	0.197	0.198	0.000	0.320
C18:1	1.63	1.63	1.65	1.66	1.67	1.67	0.005	0.029
C20:1	0.064	0.064	0.066	0.065	0.066	0.066	0.000	0.057
C18:2	0.981^{bc}	0.978^{c}	0.992^{ab}	0.996^{a}	0.993^{a}	0.996^{a}	0.001	<0.001
C18:3n-3	0.067^{ab}	0.066^{b}	0.068^{ab}	0.068^{ab}	0.070^{a}	0.069^{ab}	0.000	0.017
SFAs	1.17	1.12	1.19	1.15	1.17	1.20	0.009	0.079
MUFAs	1.89	1.89	1.92	1.92	1.93	1.93	0.006	0.030
PUFAs	1.04^{b}	1.04^{b}	1.06^{a}	1.06^{a}	1.06^{a}	1.06^{a}	0.002	<0.001

[a,b,c,d] Means with different superscripts within the same row differ significantly (P < 0.05). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids.

Table 6
Effect of the experimental treatments on the erythrogram and leukogram of broiler chickens.

Parameters	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	**2 g/kg**	**3 g/kg**			
Erythrogram								
RBCs (10^{6}/μL−1)	4.13^{ab}	2.82^{b}	4.77^{a}	4.52^{a}	4.13^{ab}	3.48^{ab}	0.212	0.014
Hb g dL−1)	11.88^{abc}	9.04^{c}	12.69^{ab}	13.73^{a}	12.77^{ab}	10.19^{bc}	0.514	0.006
PCV%	34.83^{ab}	26.88^{c}	36.57^{a}	37.55^{a}	36.69^{a}	29.89^{bc}	1.23	0.002
Platelets	154.50	154.50	200.00	205.00	197.00	177.50	9.53	0.525
MCV (fl)	84.41	95.19	77.04	83.41	88.75	86.00	1.90	0.072
MCH (pg)	28.80	31.99	26.71	30.42	30.90	29.28	0.575	0.050
MCHC (%)	34.11	33.61	34.69	36.55	34.82	34.06	0.349	0.161
Leukogram								
WBCs (10^{3} μL−1)	19.80^{ab}	17.12^{bc}	17.91^{abc}	16.13^{c}	19.06^{ab}	20.14^{a}	0.460	0.007
Lymphocytes (10^{3} μL−1)	14.53^{a}	10.16^{c}	12.63^{ab}	11.68^{bc}	13.68^{ab}	14.24^{a}	0.477	0.001
Heterophils (10^{3} μL−1)	3.55^{b}	5.64^{a}	3.80^{b}	3.03^{b}	3.64^{b}	3.93^{b}	0.253	0.001
Monocytes (10^{3} μL−1)	0.97^{a}	0.69^{b}	0.69^{b}	0.68^{b}	0.86^{ab}	1.00^{a}	0.042	0.003
Eosinophils (10^{3} μL−1)	0.47	0.45	0.42	0.41	0.49	0.56	0.022	0.464
Basophils (10^{3} μL−1)	0.29	0.17	0.36	0.32	0.38	0.40	0.027	0.089

[a,b,c,d] Means with different superscripts within the same row differ significantly (P < 0.05). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, RBCs: Red blood cells, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBCs: white blood cells.

comparable between the FEO-supplemented groups and the HSC, with the lowest numerical value observed in the group fed 2 g FEO/kg. Lymphocyte counts increased in the 1 and 3 g FEO/kg and paracetamol-supplemented groups, while remaining comparable between the 2 g FEO/kg group and the HSC ($P = 0.001$). Heterophil counts were lower in all FEO- and paracetamol-supplemented groups compared to the HSC ($P = 0.001$). Monocyte counts were comparable between FEO-supplemented groups and the HSC, while eosinophil and basophil counts were not significantly affected by FEO supplementation.

3.4. Serum biochemistry

Table 7 summarizes the impacts of dietary FEO supplementation on the serum thyroid hormone levels and lipid profile. Birds given 3 g FEO/kg exhibited the higher levels of T_3 and T_4 compared to the HSC ($P < 0.001$). TC levels were reduced in birds fed with 2 and 3 g FEO/kg and in the paracetamol group compared with the HSC (P < 0.001). However, LDL-C levels increased in birds fed 1 g FEO/kg relative to the HSC and paracetamol group (P < 0.001). VLDL-C and TG levels were lower in birds fed 1–3 g FEO/kg and in the paracetamol compared with the HSC (P < 0.001). No significant changes were detected in HDL-C

Table 7
Effect of the experimental treatments on serum thyroid hormones and lipid profile of broiler chickens.

Parameters	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
T_3 (ng/mL)	2.39^{c}	2.63^{bc}	3.19^{abc}	3.35^{ab}	3.97^{a}	3.59^{a}	0.147	<0.001
T_4 (ng/mL)	18.64^{d}	18.73^{d}	20.57^{c}	24.12^{ab}	25.81^{a}	22.45^{b}	0.720	<0.001
TC, (mmol/L)	4.03^{a}	3.71^{ab}	3.33^{bc}	3.22^{c}	3.20^{c}	3.22^{c}	0.083	<0.001
HDL, (mmol/l)	0.250	0.226	0.196	0.253	0.216	0.206	0.007	0.050
LDL, (mmol/l)	2.01^{c}	2.12^{bc}	2.31^{a}	2.13^{bc}	2.20^{ab}	2.17^{b}	0.024	<0.001
VLDL, (mmol/l)	1.77^{a}	1.36^{a}	0.82^{b}	0.84^{b}	0.78^{b}	0.84^{b}	0.096	<0.001
TG, (mmol/l)	1.38^{a}	1.32^{a}	1.12^{b}	1.18^{b}	1.13^{b}	1.15^{b}	0.025	<0.001

[a,b,c,d] Means with different superscripts within the same row differ significantly (P < 0.05). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, T_3: triiodothyronine, T_4: thyroxin, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very low-density lipoprotein cholesterol.

levels among groups.

3.5. Immune markers and antioxidant status

The impacts of the experimental treatments on immunological parameters and HSP gene expression are summarized in Table 8. Lysozyme activity differed among groups ($P < 0.001$) and was higher in birds fed 2 and 3 g FEO/kg versus HSC, with the highest level observed at 3 g FEO/kg. Serum concentrations of IL-10 and C3 were higher ($P < 0.001$) in birds fed 1–3 g FEO/g and paracetamol supplemented group relative to HSC. Birds fed 1–3 g FEO/kg, and those receiving paracetamol had higher phagocytic percentage and phagocytic index than HSC ($P < 0.001$). At the molecular level, the relative mRNA expression levels of HSP70, HSP90-α, and HSP90-β mRNA were significantly increased ($P < 0.001$) in birds fed 3 g FEO/kg relative to the HSC.

3.6. Immunohistochemical analysis

Fig. 1andFig. 2 show photomicrographs of splenic tissues immunoassayed with monoclonal antibodies targeting $CD3^+$T and $CD20^+$B lymphocytes, accompanied by morphometric data. Examined sections from chicken's spleen immunostained by specific monoclonal antibodies against $CD3^+$T lymphocytes surface receptor antigen demonstrated 2–3, zero, zero, 0.5–1, zero, and zero % positivity to the used marker in the TNC, HSC, FEO1, FEO2, FEO3, and paracetamol groups, respectively. Cellular cytoplasm showed moderate intensities of staining reaction. Sections immunostained against spleen $CD20^+$B lymphocytes showed 19–24, 25–30, 30–35, 45–50, 50–55, and 3–5 % in the TNC, HSC, FEO1, FEO2, FEO3, and paracetamol groups, respectively, with a moderate cytoplasmic staining reaction.

3.7. Histomorphological examination of the spleen

Fig. 3 shows histomorphological analysis of the spleen. Examined spleen tissues of the TNC group, showing normal splenic stroma and capsule. The spleen tissues of the HSC group showed lymphoid depletion, atrophy of the white and red pulp, and subcapsular edema. The spleen of the 1 g FEO/kg showed hemosiderosis and reticuloendothelial cell proliferation within the focal necrotic areas. The spleen tissues of the 2 g FEO/kg treatment group showed subcapsular edema and focal necrosis in the splenic parenchyma. Sero fibrinous exudate with scattered living, dead neutrophils. Examined spleen tissues of the 3 g FEO/k group showed healthy vascular sinusoids and splenic cords, and a thin capsule. Examined spleen tissues of the paracetamol group showed focal central necrosis surrounded by a few macrophages and lymphocytes.

3.8. Intestinal histomorphological examination

Table 9 and Fig. 4,Fig. 5, andFig. 6 show the histomorphological evaluation of the small intestine in broiler chickens. FEO supplementation improved various intestinal morphometric characteristics compared to the HSC. In the duodenum, VH was increased in birds fed 1–3 g FEO/kg and paracetamol relative to HSC ($P < 0.001$). A higher VW was recorded in birds fed 1 and 3 FEO/kg and paracetamol compared to HSC ($P < 0.001$). Both CD and muscle mass were higher in birds fed 2 and 3 g FEO/kg and paracetamol compared to the HSC ($P < 0.001$), while VH: CD was increased only in birds fed 1 g FEO/kg than the HSC ($P < 0.001$). In the jejunum, VW and CD were higher in birds fed 2 and/or 3 g FEO/kg compared to the HSC ($P = 0.006$). However, among groups, no significant changes were detected in jejunal VH, VH:CD, and muscle mass ($P > 0.05$). In the ileum, VH and VW were higher in birds fed 2 and/or 3 g FEO/kg compared to the HSC ($P < 0.05$). Birds fed 1–3 g FEO/kg increased CD and decreased VH: CD relative to the HSC ($P < 0.001$). Muscle mass was increased in birds fed 1 and 3 g FEO/kg relative to the HSC ($P < 0.001$).

3.9. Economic efficiency

The findings for the assessed economic efficiency metrics are shown in Table 10. Feed and total costs differed among groups ($P < 0.001$), and were reduced in the FO-supplemented groups by 2.0–3.4% and 1.1–2.1%, respectively, compared with the HSC. When compared with the paracetamol-supplemented group, feed and total consts were also lower in the FEO-supplemented groups by 1.4–2.7% and 1.1 –2.1 %, respectively. Relative to the HSC, significant increases ($P < 0.001$) were detected in the total return (2.0–3.2%), net profit (7.1–9.6%), economic efficiency (9.1–11.8%), and performance index (11.9–12.8%). In addition, feed cost per kg BWG was reduced in the FO-supplemented groups by 7.6– 8.9% compared with HSC ($P < 0.001$).

4. Discussion

High temperatures drastically affect broiler performance, resulting in reductions in growth rate, production index, and economic efficiency owing to the physiological impacts of HS (Rehman et al., 2018). The current study revealed no significant differences in growth performance among treatment groups during the starter phase, indicating that early development in broiler chickens is not notably influenced by FEO or paracetamol supplementation. Given that the starter phase was under standard ambient temperature, these observations may indicate the limited metabolic requirements of bioactive compounds or specific pharmaceuticals under standard environmental conditions. However, during the grower phase, growth performance indices were reduced in all groups supplemented with different levels of FEO and in the paracetamol-supplemented group. The current study applied HS from d 22 to 25, indicating that the birds experienced oxidative stress during the last four days of the grower period. This stress likely led to the observed decreases in FI and BW at the end of this period (Table 4). Interestingly, the improved FCR in the HSC suggests that removing oxidative stressors (i.e., by pharmaceutical treatments like paracetamol) may not always have advantageous performance, underscoring the possible need for supplementary interventions. During the finisher

Table 8
Effect of the experimental treatments on the blood biochemical parameters, immune indices, and antioxidant status of broiler chickens.

Parameters	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
Lysozyme (μg/mL)	127.66^{c}	129.00^{c}	133.66^{c}	142.33^{b}	151.00^{a}	129.66^{c}	2.22	<0.001
IL-10 (pg/mL)	1.30^{c}	1.50^{c}	2.30^{b}	3.06^{ab}	3.53^{a}	2.63^{b}	0.219	<0.001
C3 (mg/dL)	1.05^{c}	1.08^{c}	1.19^{b}	1.29^{a}	1.33^{a}	1.19^{b}	0.027	<0.001
Phagocytic %	56.50^{c}	49.00^{c}	74.00^{b}	83.50^{ab}	79.00^{ab}	86.00^{a}	4.204	<0.001
Phagocytic index	2.32^{bc}	0.96^{c}	3.32^{ab}	4.44^{a}	3.71^{ab}	4.61^{a}	0.390	<0.001
HSP70 mRNA	1.00^{c}	1.00^{c}	1.73b^{c}	2.43^{b}	3.36^{a}	2.30^{b}	0.213	<0.001
HSP90-α mRNA	1.00^{d}	1.00^{d}	1.26^{cd}	2.03^{b}	2.73^{a}	1.80^{bc}	0.162	<0.001
HSP90-β mRNA	1.00^{c}	1.00^{c}	1.70^{ab}	1.90^{ab}	2.06^{a}	1.63^{b}	0.107	<0.001

[a,b,c,d] Means with different superscripts within the same row differ significantly ($P < 0.05$). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, IL-10: interleukin-10, C3: complement3, HSP70: heat shock protein70, HSP90-α: heat shock protein-α, HSP90-β: heat shock protein90-β

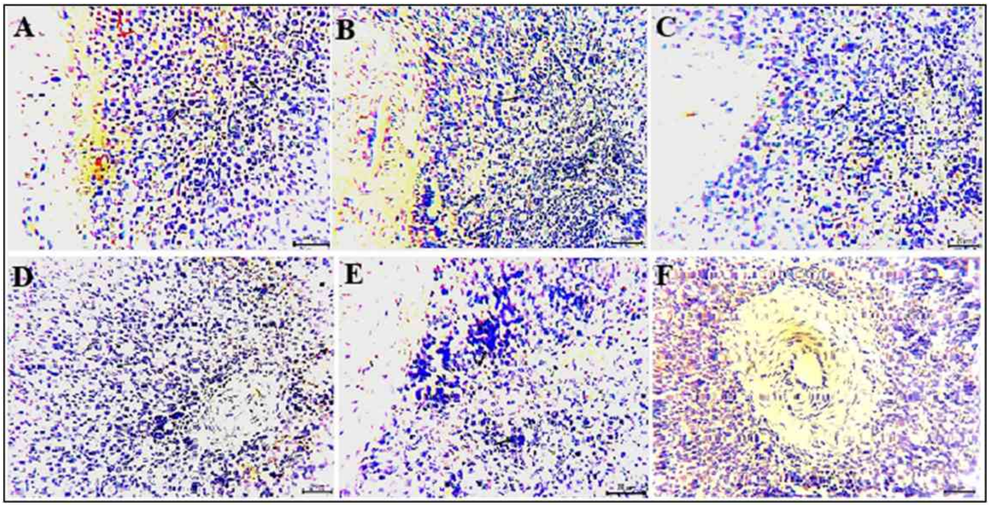

Fig. 1. Photomicrographs from the chicken's spleen immunostained by a monoclonal antibody against CD3$^+$T lymphocytes surface receptor antigen showing the % of the expressed antigen as brown cytoplasmic staining reaction of moderate intensity (red arrows). Black arrows point to negative cells. A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group. Scale bars 20 μm.

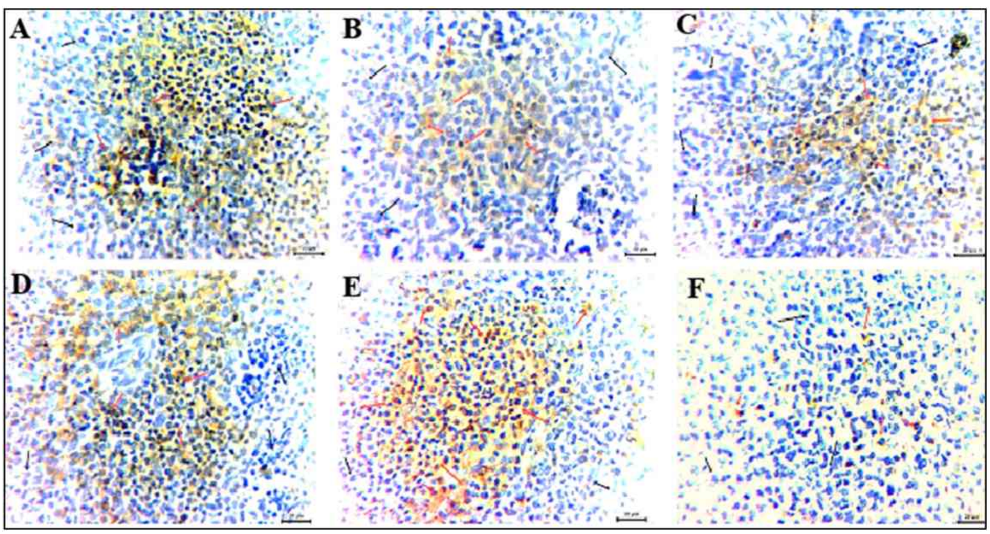

Fig. 2. Photomicrographs from a chicken's spleen immunostained by a monoclonal antibody against CD20$^+$B lymphocytes showing the % of the expressed antigen as brown cytoplasmic staining reaction of moderate intensity (red arrows). Black arrows point to negative cells. A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group. Scale bars 20 μm.

phase and overall performance, FEO supplementation improved BW and BWG relative to the HSC, demonstrating protective or rehabilitative advantages of dietary FEO following exposure to HS. The results throughout the finisher phase and overall performance revealed a decrease in FI and an improvement in FCR, which may indicate effective nutrient utilization and thermoregulatory adaptation attributed to diminished oxidative stress and improved metabolic efficiency.

The GC-MS analysis which was conducted in the current study identified anethole (17.44%), estragole (16.57%), D-limonene (13.41%), 9-octadecenoic acid (Z)-, methyl ester (8.36%), and hexadecenoic acid, methyl ester (5.19%) as primary bioactive compounds of FEO. The antioxidant, anti-inflammatory, and digestive stimulant properties of these compounds are well documented (Freire et al., 2005; Özbek et al., 2003; Rather et al., 2016). The enhanced performance indices observed in the finisher and overall periods were possibly the result of the synergistic actions of these compounds, which supported oxidative balance, nutrient assimilation, and metabolic adaptation under HS conditions.

Our present findings relatively correspond with earlier research regarding the advantageous impact of fennel supplementation under HS circumstances. Fatima et al. (2022) found that crushed fennel seeds at levels of 20 and 25 g/kg enhanced BWG, FI, and FCR in broilers subjected to HS, which aligns with our current findings of improved BW and BW during the finisher and overall performance periods. Meanwhile, studies on laying hens reported that fennel supplementation at levels of 10 and 20 g/kg had little impact on performance metrics such as FI, BWG, and egg production; however, it efficiently diminished the adverse effects of HS on egg quality indices (Gharaghani et al., 2015). This may support the fact that fennel may not precisely improve growth performance metrics under stress conditions; otherwise, it may aid in mitigating stress-induced physiological disruptions. The abovementioned research employed various forms of fennel, dosages, and durations of HS exposure, affecting cross-study comparisons. Consequently, more investigation is necessary to validate dietary FEO's influence on broilers' growth performance under stress conditions.

In the current study, dietary FEO supplementation elevated PUFA

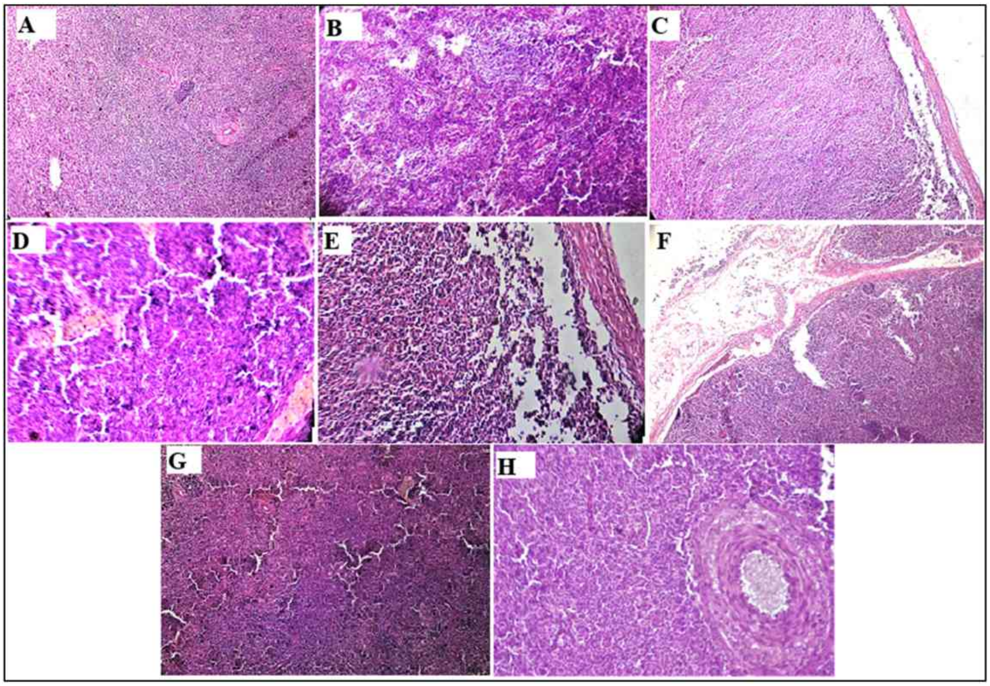

Fig. 3. Photomicrograph of H&E-stained sections of the spleen tissues in the experimental groups. A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group.

Table 9
Effect of the experimental treatments on the histomorphological measures (μm) of the small intestine of broiler chickens.

Parameter	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
Duodenum								
VH, μm	1067^{ab}	574^{c}	1224^{ab}	1310.00^{ab}	1511.00^{a}	1286.00^{ab}	76.705	<0.001
VW, μm	73^{bc}	47.3^{c}	85.3^{b}	74.33^{bc}	145.33^{a}	103.65^{b}	7.802	<0.001
CD, μm	204^{b}	99^{c}	166^{bc}	252.33^{ab}	306.33^{a}	314.00^{a}	19.751	<0.001
VH: CD	5.22^{bc}	5.78^{b}	7.43^{a}	5.20^{bc}	5.00b^{c}	4.11^{c}	0.272	<0.001
MCT, μm	182^{b}	147^{b}	188^{b}	325.67^{a}	325.33^{a}	306.33^{a}	19.01	<0.001
Jejunum								
VH, μm	908	909	1150	1283.33	1016.67	1362.00	74.58	0.383
VW, μm	75^{b}	74.6^{b}	102^{ab}	101.67^{ab}	125.00^{a}	114.00^{ab}	5.34	0.006
CD, μm	157^{bc}	143.33^{c}	204^{abc}	247.67^{ab}	255.33^{a}	229.33^{abc}	12.01	0.006
VH:CD	5.97	6.36	5.64	5.20	4.32	6.02	0.329	0.602
MCT, μm	174	140	365	205.67	271.33	224.00	29.99	0.342
Ileum								
VH, μm	527.00^{c}	512.00^{c}	617.00^{bc}	691.33^{ab}	792.00^{a}	696.67^{ab}	26.12	<0.001
VW, μm	73.00^{b}	64.67^{b}	72.33^{b}	129.33^{a}	92.67^{ab}	98.00^{ab}	5.96	0.001
CD, μm	175.00^{d}	141.33^{d}	228.00^{bc}	310.33^{a}	244.67^{b}	180.33^{cd}	13.90	<0.001
VH: CD	3.03^{c}	3.61^{ab}	2.71^{cd}	2.23^{d}	3.24b^{c}	3.87^{a}	0.138	<0.001
MCT, μm	220.67^{b}	191.67^{b}	273.00^{a}	215.00^{b}	322.00^{a}	300.67^{a}	12.12	<0.001

^{a,b,c,d} Means with different superscripts within the same row differ significantly ($P < 0.05$). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, VH: villus height, VW: villus width, CD: crypt depth, MCT: muscular coat thickness.

levels in the breast muscle of broiler chickens, underscoring its nutritional advantages under HS. Birds receiving 1–3 g FEO/kg demonstrated elevated total PUFA levels compared to the HSC, with notable increases in linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3) at 3 g FEO/kg. From a consumer standpoint, increased levels of PUFA, especially n-3 FA, correlate with enhanced nutritional quality of poultry meat due to their contribution to lowering cardiovascular disease risks (Russo, 2009; Wood et al., 2008). Notably, the levels of SFA, including palmitic acid (C16:0) and stearic acid (C18:0), were not significantly affected by FEO supplementation. This suggests that the observed enhancement in PUFA deposition in chicken breast muscle occurred without corresponding rises in SFA, which is advantageous considering the link between elevated SFA intake and human metabolic disorders (Kennedy et al., 2009; Silva Figueiredo et al., 2017). Comparable enhancements in PUFA content have been shown with other essential oils, which enhance PUFA levels in broiler chickens' serum and thigh muscle (Hashemipour et al., 2013). Interestingly, Hashemipour and colleagues did not observe alterations in the fatty acid profile of breast muscle in response to essential oils supplementation, suggesting that the current study's findings necessitate further investigation to validate these changes and elucidate the underlying mechanisms.

According to the current study, adding varying levels of FEO to broiler diets improved many hematological indices, such as RBCs count, Hb, and PCV; however, there were no significant differences in MCV, MCH, MCHC, or platelet count between groups. These results align with previous research in various mammalian species, which found that supplementing with fennel seed extract enhanced erythrocytic parameters. For example, Mansouri et al. (2015) reported increased RBCs

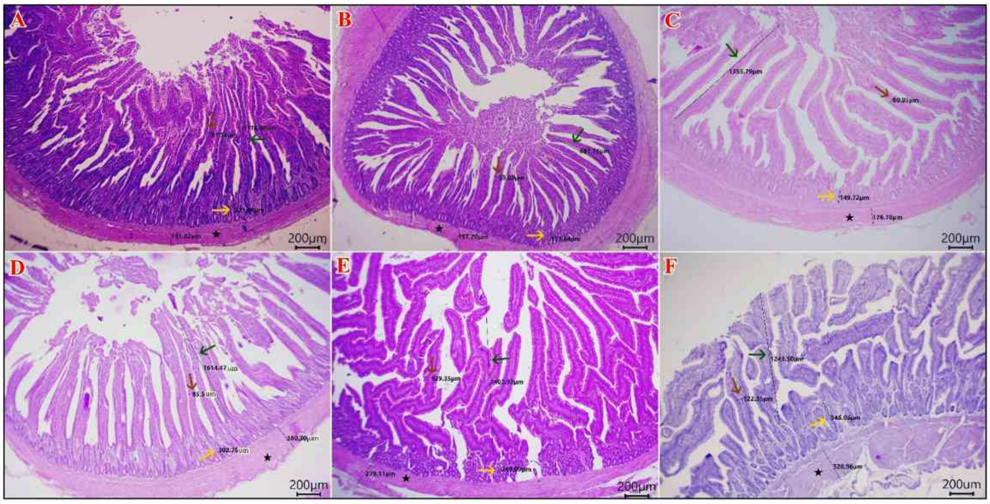

Fig. 4. Photomicrograph of H&E-stained sections of duodenum of the experimental groups showing: VH "villus height" (green arrows), VW "villus width" (brown arrow), and CD "crypt depth" (yellow arrows), and muscular coat thickness "MC" (black star). A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group. Scale bar 200 μm.

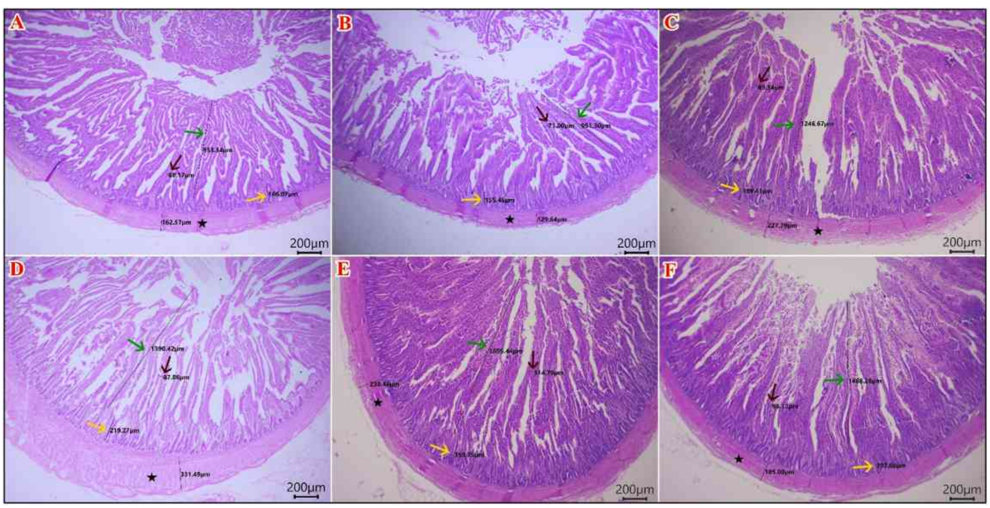

Fig. 5. Photomicrograph of H&E-stained sections of the jejunum of the experimental groups showing: VH "villus height")green arrows). VW "villus width" (brown arrow), CD "crypt depth" (yellow arrows), and muscular coat thickness "MC" (black star). A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group. Scale bar 200 μm.

count following supplementation with fennel seed extract at 250 mg/kg BW, while Abbas et al. (2021) found similar hematological improvement using 4% *Foeniculum vulgare*. To explore the hematopoietic potential of fennel, Oktay et al. (2003) showed that phenolic and aqueous extracts of fennel seeds have potent antioxidant properties, as demonstrated by metal chelation activities and anionic radicals absorption tests. These antioxidant qualities probably contribute to the longevity of RBC membranes by protecting them from oxidative stressors. The improved erythrogram indices seen in the current study could be explained by this suggested mechanism, which would also improve physiological performance and oxygen-carrying capacity, particularly in HS conditions, which are known to cause oxidative damage in poultry. Further research is necessary to determine the precise mechanisms by which FEO affects hematology, though, given the unchanged values of MCV, MCH, and MCHC, indices that primarily represent RBCs volume and Hb content per cell, rather than total cell count or overall amount of Hb in whole blood (El Brihi and Pathak, 2024).

Furthermore, the current investigation indicated that varying supplemental levels of FEO in broiler diets influenced several leukogram parameters, such as total WBCs, lymphocytes, heterophils, and monocytes; however, the counts of eosinophils and basophils remained unchanged across treatments. Notably, the lowest total WBCs count was observed in birds supplemented with 2 g FEO/kg, while WBC values in the other FEO-supplemented groups were comparable to those of the HSC. This finding disagrees with earlier research reporting increased WBC counts following dietary supplementation with the powder of fennel seeds in broilers (Khafaji, 2024); Mohammed and Abbas (2009), suggesting that differences in fennel form, inclusion level, or experimental conditions may account for the observes responses. Heat stress is known to significantly affects the leukogram profiles, specifically reducing lymphocyte and monocyte count in the blood while increasing heterophil counts, reflecting stress-induced immune response (Aengwanich and Suttajit, 2010; Ruell et al., 2014). Therefore, nutritional supplementation with phytogenic feed additives may be an effective intervention to alleviate the immunosuppressive effects of HS. Indeed, Cherng et al. (2008) demonstrated that fennel seed extract exerted immunomodulatory effects on peripheral blood mononuclear cells (PBMCs), indicating a stimulatory influence on immune cell

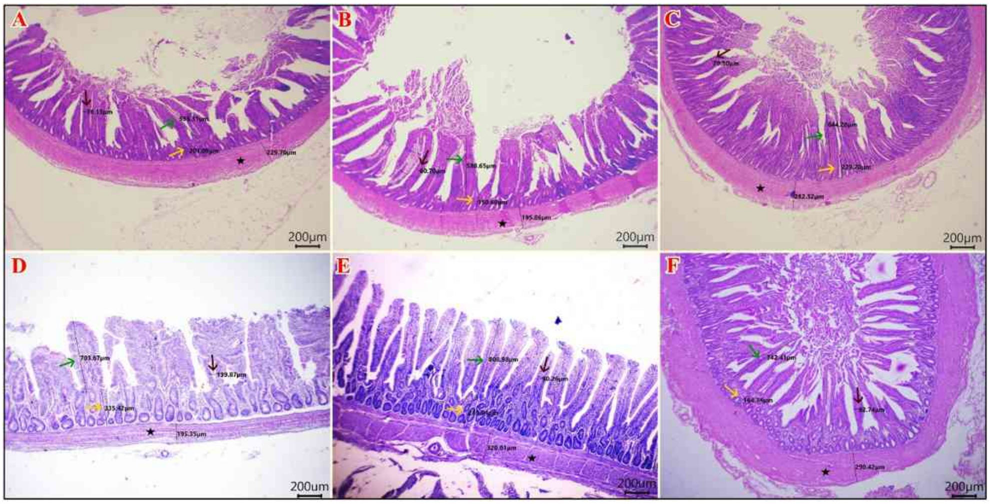

Fig. 6. Photomicrograph of H&E-stained sections of the ileum of the experimental groups showing: VH "villus height")green arrows), VW "villus width" (brown arrow), and CD "crypt depth" (yellow arrows), and muscular coat thickness "MC" (black star). A: thermoneutral control, B: heat-stressed control, C, D, and E are fennel essential oil-supplemented groups at 1, 2, and 3 g/kg, respectively, F: paracetamol group. Scale bar 200 μm.

Table 10
Effect of the experimental treatments on the economic efficiency.

Parameters	TNC	HSC	FEO			500 mg paracetamol/L	SEM	*P*-value
			1 g/kg	2 g/kg	3 g/kg			
Feed costs (USD/bird)	1.53^{a}	1.48^{ab}	1.43^{b}	1.45^{b}	1.43^{b}	1.47^{b}	0.007	<0.001
Total costs (USD/bird)	1.93^{a}	1.87^{ab}	1.83^{b}	1.85^{b}	1.83^{b}	1.87^{b}	0.007	<0.001
Total return (USD/bird)	3.87^{a}	3.43^{c}	3.54^{b}	3.53^{b}	3.50^{b}	3.48^{bc}	0.021	<0.001
Net profit (USD/bird)	1.94^{a}	1.56^{d}	1.71^{b}	1.68^{bc}	1.67^{bc}	1.61^{cd}	0.018	<0.001
Feed cost/kg gain (USD/bird)	1.33^{d}	1.57^{a}	1.45^{bc}	1.44^{bc}	1.43^{c}	1.51^{ab}	0.012	<0.001
Economic efficiency	1.01^{a}	0.833^{c}	0.931^{b}	0.911^{b}	0.909^{b}	0.863^{bc}	0.010	<0.001
Performance index	142^{a}	109^{c}	123^{b}	122^{b}	123^{b}	116^{bc}	1.69	<0.001

^{a,b,c,d} Means with different superscripts within the same row differ significantly ($P < 0.05$). TNC: thermoneutral control, HSC: heat-stressed control, FEO: fennel essential oil, Total return (USD/bird) = Live BW/bird × Price of kg BW. Net profit (USD/bird) = Total returns − total costs. Economic efficiency = Net profit/total feed cost.

activity. In chicken, PBMCs include various immune cell types, including T cells, B cells, natural killer cells, monocytes, and dendritic cells, all of which play vital roles in combating infections and inflammatory stressors (Qu et al., 2022; Tantikositruj et al., 2021). In the current study, however, supplemental FEO under HS was associated with reductions in total WBC, monocyte, and heterophil counts, which appears to contrast with earlier reports of the immune stimulatory impact of fennel extract. Despite this, lymphocyte counts were increased in birds fed 1 and 3 g FEO/kg relative to HSC, suggesting a possible protective immunomodulatory effect of FEO against stress-induced lymphocyte suppression. These divergent observations indicate that supplemental FEO in broiler diets may selectively modulate certain leukocytes under HS circumstances, necessitating further precise elucidation of the mechanisms by which dietary FEO affects the leukogram of broilers under environmental stresses.

The present investigation aimed to improve our understanding of the metabolic and immunological responses associated with dietary FEO supplementation in broiler chickens exposed to HS. Thermoregulation in poultry is a complex process involving neurological, hormonal, and immunological mechanisms. In this context, serum biochemical measurements act as significant markers to analyze these responses and estimate the heat tolerance of broiler chickens. Currently, we have seen reductions in TC, TG, and VLDL-C with dietary FEO supplementation, indicating improved lipid homeostasis and a hypolipidemic impact. Previous studies have shown that several bioactive compounds in essential oils, including fenchyl, borneol, citral, and geraniol, have a vital role in suppressing the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), a key enzyme in cholesterol synthesis (Yu et al., 1994). A correlation exists between LDL-C, TC, and HMG-CoA reductase activity in chickens, but not with HDL (Hong et al., 2012; Qureshi et al., 1983). Therefore, the hypolipidemic effect of FEO in the present study may, at least in part, be mediated through modulation of hepatic cholesterol synthesis. To the best of our knowledge, no earlier studies have specifically investigated the role of trans-anethole, the major phenylpropanoid in FEO, in regulating lipid metabolism of broiler chickens. Intriguingly, Ghiasvand et al. (2021) reported no significant changes in serum TG, TC, and LDL-C in broilers supplemented with FEO in corn or wheat-based diet, indicating that diet composition, HS conditions, and FEO dosage may influence the dietary FEO impact on broilers' lipid profile. Further mechanistic studies are required to investigate whether trans-anethole or other FEO components impact lipid metabolism in poultry, particularly under stressful conditions.

Our findings demonstrated that dietary supplemental FEO, particularly at a level of 3 g/kg, significantly raised thyroid hormone levels, improved immune response indices, and upregulated the expression of HSP70, HSP90-α, and-β in heat-stressed birds. The observed elevation in serum T_3 and T_4 levels in the group administered 3 g FEO/kg is consistent with prior research by Nassar et al. (2024), who reported higher plasma T_3 levels in response to fennel seed powder supplementation at 30 g/kg under thermoneutral conditions. It is well established that thyroid gland function is intimately related to oxidative status; prior research has documented significant oxidative damage and diminished antioxidant defense mechanisms in thyroid tissue during hormone synthesis (Landex et al., 2006). Additionally, Yahav (1999) found that heat-stressed chickens have lower thyroid hormone levels because of decreased glandular activity, which is inversely connected with ambient temperature and linked to a drop in metabolic rate. Our findings imply that adding FEO to broiler diets may effectively protect the thyroid gland in HS conditions by reducing oxidative damage and promoting endocrine function.

With the highest effects observed at the 3 g FEO/kg level, our results show that dietary supplementation with FEO had improved the immune status of heat-stressed broiler chickens. It is well known that HS disturbs the immune system in chickens, causing various immune-related tissues to release immunological biomarkers, such as pro-and anti-inflammatory cytokines. In the current study, higher serum lysozyme activity, IL-10, and C3 concentrations, as well as higher phagocytic percentage and phagocytic index, provide an indication that dietary FEO supplementation considerably modulated immune response under HS conditions. Furthermore, immunohistochemical analysis revealed a marked rise in $CD20^{+}$B- lymphocyte immunostaining in splenic tissue. In parallel, cellular immune response was also modulated in response to dietary FEO, as shown by the elevated $CD3^{+}$T-lymphocyte immunostaining in the spleen, particularly at the dose of 2 g/kg. Interleukin-10 is a crucial anti-inflammatory cytokine that modulates the immune response under various pathophysiological conditions (Herrero et al., 2003; Yu et al., 2009). Whereas, complement 3, on the other hand, has been documented to initiate inflammation as a defensive response to external invaders (Dunkelberger and Song, 2010). lysozymes is likewise recognized for their role in eradicating intestinal infections in poultry through multiple mechanisms, including the enhancement of macrophages' phagocytic activities (Saurabh and Sahoo, 2008). Lymphocyte subpopulations are distinguished by distinct cell surface markers that regulate immune activation. Upon antigen identification, the T-cell co-receptor CD3 triggers a signaling cascade that activates both helper and cytotoxic T cells (Ryan, 2010), whereas the B-cell surface antigen CD20 modulates B-cell activity, differentiation, and proliferation (Tedder and Engel, 1994). Thus far, our results suggest that dietary FEO supports both humoral and cell-mediated immunity, providing a thorough immunomodulatory effect that may alleviate the detrimental effects of HS in poultry. These observations are consistent with earlier research demonstrating the immunomodulatory effect of fennel supplementation in broiler diets. After adding fennel seed powder (30 g/kg) and FEO (200 mg/kg) to the diet, Nassar et al. (2024) and Mirzaei et al. (2023) observed improved cellular and humoral-mediated immune responses, as demonstrated by increased antibody titers against sheep RBCs and improved phytohemagglutinin-wattle reaction. Similarly, Fatima et al. (2022) showed that broiler chickens fed fennel seed powder under HS conditions had significantly higher antibody titers against the Newcastle disease virus. These immunological benefits are likely attributed to fennel's bioactive compounds and antioxidant properties, which may protect immune cells from oxidative damage and promote cellular proliferation, particularly in oxidative and thermal stress (Ma et al., 2005).

Histological examination of the spleen in the current study revealed further verification of the positive effect of dietary FEO supplementation under HS conditions. The HSC group showed HS-induced lesions in the spleen, characterized by lymphoid depletion, atrophy of the white and red pulp, and subcapsular edema, indicative of compromised splenic architecture in heat-stressed broilers (Chen et al., 2024; Hirakawa et al., 2020). Interestingly, intermediate doses of FEO could not completely restore splenic morphology under HS; however, dietary FEO supplementation at 3 g/kg exhibited splenic integrity, in terms of healthy vascular sinusoids, splenic cords, and a thin capsule. This suggests that elevated dietary FEO dose could mitigate HS's harmful impact, presumably due to the antioxidant properties of its bioactive components (i.e., trans-anethole) (Abd El-Hack et al., 2022; Zhang et al., 2018).

Animals produce more HSPs in reaction to HS as a defense mechanism. HSPs are kept at low basal concentrations under normal physiological conditions; nevertheless, exposure to environmental stressors, like high temperatures, causes them to be significantly expressed, which helps to mitigate stress-induced cellular damage (Flees et al., 2017). By keeping cytoskeletal and other cellular proteins stable, HSPs provide protection, especially in oxidative stress situations where ROS can cause denaturation (Duchateau et al., 2020). Among the HSP family, HSP70 is the most extensively investigated owing to its potential protective attributes against various stresses (Gabriel et al., 2002). Similarly, HSP90 expression has been shown to rise rapidly in broiler chickens following HS exposure, with elevated levels detected within 2 h across multiple tissues (Lei et al., 2009). Exposure to high ambient temperatures ranging from 37 to 42 °C can increase HSP90 expression by up to twofold, which helps cellular homeostasis and an efficient stress response (Bagatell et al., 2000; Jackson, 2012). In the present study, the hypothesis that FEO provides cellular protection under HS conditions was supported by the observed upregulation of hepatic HSP70, HSP90-α, and HSP90-β expression in chickens fed dietary FEO supplementation.

Dietary FEO supplementation influenced intestinal morphology in broiler chickens, particularly by improving VH, VW, and muscle thickness in different parts of the small intestine. The small intestine is essential for the digestion and absorption of nutrients, and its functional capacity is often assessed by morphometric metrics such as VH, VW, CD, and muscle thickness (Gao et al., 2008). Increased VH and VW, muscle thickness, and shallower CD are generally associated with improved absorptive surface area (Zeitz et al., 2015). In the current study, duodenal and ileal VH, VW, and muscle thickness were elevated at various supplementary dosages of FEO. At the same time, in the jejunum, only VW increased at the highest inclusion level (3 g FEO/kg). Longer and wider villi enhance the intestinal surface area, which is essential for optimizing nutrition absorption and facilitating better growth performance in broiler chickens. Moreover, an augmented intestinal surface area may indicate increased intestinal mass and length, further improving nutrition absorption and utilization capacity (Yamauchi, 2002). A reduction in CD in the current study was not observed at varying inclusion levels of FEO. Intestinal crypts are where stem cells proliferate to facilitate villus renewal (Potten, 1998; Uni et al., 1998). Increased CD is generally associated with accelerated tissue turnover and a heightened demand for tissue regeneration, often in response to stress or damage (Miles et al., 2006; Xia et al., 2004). Conversely, shallower crypts suggest the prolonged survival of villi without renewal, which may reflect improved intestinal health and stability, reflecting reduced regenerative pressure and lower energy expenditure allocated to intestinal maintenance, allowing more energy to be redirected toward growth and productive functions (Biasato et al., 2018; Pluske et al., 1997). The lack of substantial decreases in CD in our study may indicate that, although FEO promotes villus development, it does not consistently influence epithelial regeneration processes.

Our findings reveal partial agreement with earlier studies. Ghiasvand et al. (2021) showed that 200 mg FEO/kg increased ileal VW and surface area while reducing muscle and lamina propria thickness in broilers. İpçak et al. (2024) also demonstrated that dietary FEO improved small intestine morphology, including VH, VW, and the thickness of the mucosal and muscular layers, with the most significant effects shown at 200 and 400 mg/kg. The partial inconsistency in the findings between our study and previous work may be attributed to differences in FEO inclusion levels, experimental design, or environmental stressors. Overall, the variable effects of FEO on various intestinal metrics and segments, especially the absence of improvement in CD despite increases in VH, VW, and muscle thickness, underscore the complexity of its mechanism of action. The inconsistencies and differing results in previous investigations highlight the necessity for more research to elucidate the dose-dependent and segment-specific effects of FEO on intestinal development in broiler chickens.

From an economic standpoint, FEO supplementation in broiler diets reduced feed and overall costs, while improving profitability indicators relative to the control. These observations indicate that dietary FEO markedly enhances production efficiency, particularly under HS circumstances. The reduced feed and overall expenses in FEO-supplemented groups can be attributed to improved growth performance and feed efficiency throughout the finisher phase and over the entire experimental period, as indicated by the decreased feed cost per kg of BWG. This suggests that birds consuming FEO efficiently utilized the feed and convert it into body weight, thereby reducing overall production costs. Moreover, the significant enhancement in total return, net profit, economic efficiency, and performance index in FEO-supplemented groups compared to the HSC further supports its economic benefits under HS settings. To our knowledge, limited information is available regarding the economic efficiency of FEO supplementation in broiler diets. However, comparable results were observed by Wade et al. (2018), who reported enhanced economic performance indicated by increased profit per kg of live BW in response to dietary supplementation of thyme essential oil in broiler chickens. Collectively, the bioactive components and antioxidant properties of dietary essential oils, which reduce oxidative stress and support better health under HS conditions, are likely responsible for the increased economic performance of these oils (Abd El-Hack et al., 2022).

5. Conclusions

In summary, dietary supplementation with 1–3 g/kg FEO improved overall growth performance and economic efficiency of broiler chickens under HS, as indicated by improved BW, BWG, FCR, and economic efficiency. Also, FEO supplementation positively modulated selected hematological indices (i.e., RBCs count, HB, and PCV), fatty acid profile of breast muscle, serum lipid profile, thyroid hormone levels, immune response, hepatic HSPs expression, and intestinal and splenic histomorphlogy, with the most consistent responses generally observed at 3 g/kg. Variable leukogram measures indicate a selective modulation of leukocyte populations under HS. Collectively, these findings indicate that FEO may serve as a potential phytogenic strategy for mitigating the negative effects of HS in broiler chickens. Nevertheless, further investigation is necessary to refine dosing and understand bioavailability across different rearing stages and environmental conditions.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Grammarly to check grammar and paraphrase the sentences where needed and ENDNOTE for reference citation. After using this tool/service, the author(s) reviewed and edited the content as required and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Samar A. Tolba: Data curation, Methodology, Software, Writing – original draft, Writing – review & editing. **Shimaa A. Amer:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Abdel-Wahab A. Abdel-Warith:** Investigation, Software, Writing – review & editing. **Elsayed M. Younis:** Investigation, Software, Writing – review & editing. **Ahmed Gouda:** Data curation, Methodology, Writing – review & editing. **Gehad E. Elshopakey:** Methodology, Writing – review & editing. **Noura A. Abd-Allah:** Methodology, Writing – review & editing. **Rehab Reda:** Methodology, Writing – review & editing. **Rehab I. Hamed:** Data curation, Methodology, Supervision. **Gehan K. Saleh:** Investigation, Methodology. **Gehan N. Alagmy:** Methodology, Software. **Simon J. Davies:** Writing – review & editing. **Fatma Salih Mohammed:** Methodology, Writing – review & editing.

Declaration of competing interest

The authors declared no conflict of interest.

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Data availability

The authors do not have permission to share data.

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