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Evaluation of adding tea tree oil and walnut oil on production and physiological performance of broiler chickens under high stock density breeding

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Abstract. Dietary supplementation of poultry diets with essential oils is a natural strategy to mitigate stress and enhance the quality of poultry products. This study investigated the impact of tea tree oil (TOL) or walnut oil (WO), alone and in combination, on performance, carcass traits and quality, blood chemistry, and antioxidant activity in broilers reared under normal stocking density (NSD) and high stocking density (HSD) conditions. A total of 520 d-old Cobb 500 broiler chicks were randomly distributed to eight groups according to a 2×4 factorial design. All chicks were randomly allocated to eight experimental groups, with five replicates per group. Each replicate consisted of 16 birds in HSD groups and 10 birds in NSD groups. The two factors were stocking densities (16 and 10 birds m^{-2}) and four levels of feed additives: control (no oil), TOL (5 mL TOL kg^{-1} diet), WO (5 mL WO kg^{-1} diet), and combination (5 mL TOL + 5 mL WO kg^{-1} diet). There were significant interactions between treatments and stocking densities for performance criteria (average weight gain (AWG), feed intake (FI), and feed conversion ratio (FCR)), carcass traits (dressing percentage, breast, and thigh yield), and blood metabolites (glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and antioxidant activity, as well as omega-3 and the ratio of omega-3 to omega-6. Dietary WO significantly increased final body weight and AWG compared with TOL and the control group at both stocking densities ($P < 0.05$). Birds reared under HSD fed with dietary additives WO, TO, and the combination had lower FCR than NSD birds ($P < 0.05$). Overall, these results elucidate the beneficial effects of individual supplementation with WO or TOL in enhancing the performance and health status of broilers exposed to HSD. However, the combination of these oils did not exhibit any synergistic effects.

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1 Introduction

Rising living standards have increased consumer demand for high-quality meat with desirable nutritional, sensory, and safety characteristics (Yan et al., 2021). To meet this demand, the poultry industry has intensified production by increasing the number of birds confined in small spaces. Although intensive production systems improve space-use efficiency, excessive stocking density can impair broiler growth performance, health, and welfare, and increase disease pressure (Estevez, 2007). High stocking density (HSD) in broilers can adversely affect production by reducing feed intake (FI) and body weight gain (BWG), and increasing feed conversion ratio (FCR) (Li et al., 2019; McKeith et al., 2020; Son et al., 2022; Thema et al., 2022). These effects may be associated with increased competition for feeder space. In addition, HSD reduced carcass and meat quality, while increasing litter moisture and foot pad score (Dozier et al., 2005; Abdallah et al., 2024). High litter moisture increases bacterial loads and contributes to several enteric diseases (Wilkinson et al., 2011). In conventional poultry production systems, antibiotics were used at subtherapeutic levels to maintain production and improve gut health. However, indiscriminate use of antibiotics causes bacterial resistance (Paul et al., 2022), and consumer demand for antibiotic-free animal products has also led to a decrease in the use of antibiotics in animal production settings (Thaxton et al., 2016). Consequently, the European Union banned the use of antibiotics as growth promoters in animal feed production in 2006. Consequently, researchers have been looking for nutritional alternatives (Julean et al., 2021). Plant-derived essential oils are one promising approach because of their antioxidant, antimicrobial, and digestion-enhancing properties (Mansour et al., 2025), which may support poultry health, performance, and the nutritional composition of meat. Great attention has been directed toward human diets containing polyunsaturated fatty acids (PUFAs), such as omega-3 and omega-6, because of their beneficial roles in preventing cardiovascular diseases by lowering blood cholesterol and autoimmune diseases (WHO, 2003; O'Neill and Minihane, 2017; Alagawany et al., 2018, 2021). Chicken meat is a potential source of animal protein with a high concentration of PUFA (Soriano-Santos, 2010). Accordingly, dietary supplementation with seed oil, vegetable oils, and fish oil has been investigated as a strategy to enrich poultry meat with PUFAs and improve its nutritional value (Konieczka et al., 2017; Madkour et al., 2025). Among seed oils, walnut oil (WO) (*Juglans regia* L.) has one of the highest amounts of PUFA (up to 78 % of the total FA content). It contains linoleic acid (omega-6) 54 %–57 %, linolenic acid (omega-3) 14 %–15 %, oleic acid (omega-9) 16 %–17 %, and palmitic acid 7 %–8 % (Aryapak and Ziarati, 2014; Elouafy et al., 2022). Walnut oils were selected for their high content of omega-3 PUFAs and their potential to enhance the nutritive value of meat products (Stamenić et al., 2025; Taku et al., 2025). Therefore, WO may be a good choice to improve the nutritional quality of broilers.

Tea tree oil (TOL) is an essential oil extracted from the *Melaleuca alternifolia* plant, and it is native to the Australian region (Delves-Broughton, 2012). TOL contains more than 100 different compounds, mainly cyclic monoterpenes (Yang et al., 2022), and also contains terpinene-4-ol (39.8 %), α-terpinene, β-terpinene, α-terpineolene, γ-terpinene, and 1, 8-cineole, which are considered the major active antimicrobial and antioxidant components of TOL (Puvača et al., 2019). In addition, TOL can stimulate lymphocyte proliferation and suppress pro-inflammatory cytokines, thereby manifesting anti-inflammatory activity (Brand et al., 2002). There are considerable studies providing strong evidence on the positive effects of TOL, but fewer studies have confirmed its ability to enhance the growth performance of broiler chickens (Julean et al., 2021; Abo Ghanima et al., 2021).

High stocking density can increase environmental and physiological stress in broiler chickens, potentially impairing their growth, health, and welfare (Estevez, 2007; Sugiharto, 2022). These challenges underscore the need to investigate nutritional strategies that could help to sustain productivity under intensive rearing conditions. The TOL and WO were selected due to their potential complementary biological properties. TOL contains some bioactive compounds with antioxidant and antimicrobial potential, whereas WO is a source of UFA and other bioactive constituents that may improve antioxidant defenses and support lipid metabolism (Stamenić et al., 2025; Taku et al., 2025). These complementary properties provide a rationale for investigating the oils as dietary supplements under different stocking densities. However, whether TOL and WO alone and in combination can improve performance and physiological responses under different stocking densities remains unclear. Therefore, this study evaluated the effects of adding TOL and/or WO, individually and in combination, to broiler feed on production and physiological changes in broiler chickens reared under high and normal stocking densities. The 2×4 factorial design also enabled us to examine potential interactions between dietary supplementation and stocking density.

2 Materials and methods

2.1 Animal welfare and ethical approval

The procedures of the experiment were approved by the Ethical Committee of the Faculty of Agriculture, Assiut University, Egypt (no. 2023/10/01) regarding the protection of animals used for scientific purposes. The research adhered to the International Health Organization's rules, Institutional Animal Care and Use Committee (IACUC), the Helsinki Declaration, Good Medical and Laboratory Practice, recommendations, and relevant Egyptian legislation. European Union (EU) rules were observed in the care and use of the ani-

Table 1. Feed ingredients and nutrient levels of basal diets.

Ingredient (%)	Starter (0–14 d)	Grower (15–28 d)	Finisher (29–35 d)
Yellow corn	52.27	59.10	63.20
Soybean meal (44 % CP)	34.01	26.68	22.49
Corn gluten (60 % CP)	6.00	7.00	6.31
Sunflower oil	3.00	3.00	4.00
Di-calcium phosphate	1.83	1.63	1.57
Lime stone	1.44	1.19	1.10
L-lysine HCl	0.33	0.33	0.28
Dl-methionine	0.27	0.21	0.17
Sodium chloride	0.23	0.23	0.25
Sodium bicarbonate	0.22	0.23	0.23
Vitamin premix[a]	0.10	0.10	0.10
Mineral premix[b]	0.30	0.30	0.30
Nutrient levels %			
Crude protein	23.17	21.25	19.14
Metabolizable energy (Kcal kg^{-1})	3100	3110	3205
Crude fiber	3.81	3.46	3.23
Crude fat	5.56	5.77	5.86
Calcium	1.04	0.90	0.84
Av. phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54

[a] Supplied per kg of diet: Vit. A, 11 000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3 mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6, 3 mg; B12, 14 mcg; nicotinic acid 60 mg; folic acid 1.75 mg, pantothenic acid 13 mg; and biotin 120 mcg. [b] Supplied per kg of diet: choline 600 mg; copper 16 mg; iron 40 mg; manganese 120 mg; zinc 100 mg; selenium 0.20 mg; and iodine 1.25 mg.

mals, including the ARRIVE guidelines 2010/63/EU (https://arriveguidelines.org/, last access: 23 November 2026).

2.2 Birds, experimental design, diets, and management

A total of 520 mixed-sex 1 d-old broiler chicks (Cobb 500) were obtained from a commercial hatchery with an average initial body weight of 42.18 ± 1.3 g $chick^{-1}$. All chicks were randomly allocated to eight experimental groups, with five replicates per group. Each replicate consisted of 16 birds in HSD groups and 10 birds in NSD groups. Birds were reared for 35 d. Birds were allocated to eight treatment groups in a 2×4 factorial arrangement, including two levels of stocking density (16 and 10 birds m^{-2}) and four dietary treatments: control (no oil), TOL (5 mL TOL kg^{-1} diet), WO (5 mL WO kg^{-1} diet), and combination (5 mL TOL + 5 mL WO kg^{-1} diet).

Commercial TOL and WO were used in this study, procured from the market (Organic Natural Oils company, Egypt). The required small amount (5 mL kg^{-1}) was blended thoroughly with sunflower oil to increase its volume and ensure uniform distribution. This premix (sunflower oil with herbal oils) was then gradually added to a larger portion of the normal diet and mixed with a mechanical mixer before being incorporated into the whole batch of feed. To maintain dietary uniformity, an equal amount of carrier oil (sunflower oil) was added to the basal diet (control), ensuring that all diets contained the same total lipid proportion. All birds were fed commercial pelleted diets for starter (d 0 to 14), grower (d 15 to 28), and finisher (d 29 to 35) periods (Table 1).

The ambient temperature was set at 33 °C during the first 3 d of age, then gradually lowered by 2–3 °C per week until the chicks were 5 weeks old. The birds were reared at a relative humidity of 56 %–66 %. Feed and fresh water were provided to chicks ad libitum throughout the entire trial period. Manual feeding and drinking systems were equipped in all pens. The birds were vaccinated according to the vaccination routine followed at the farm without any addition of antibiotics. The birds were exposed to 24 h of light on day 1, then 1 h less each day until they had 18 h of light, which was maintained until the completion of the experiment.

2.3 Growth performance, characteristics of carcass, and relative organ weights of broilers

Body weight (BW) was determined at the beginning and end of each week, and the average body weight gain (ABWG) was calculated by subtracting the initial average body weight (IABW) from the final average body weight (FABW) for each replicate. Feed intake (FI) was measured weekly for each replicate by recording the amount of diet offered and the residual diet at the end of each week. The feed conver-

sion ratio (FCR) was evaluated according to the following formula: FCR = FI/ABWG.

Mortality rates were recorded during experimental periods. At 35 d of age, after fasting for 8 h before slaughter, two birds per replicate were randomly selected (five birds for each treatment). They were individually weighed and euthanized, followed by bleeding, de-feathering, and evisceration. Carcass, cut parts (breast, thighs, wings, and neck), organs (liver, heart, spleen, and gizzard), and abdominal fat were weighed and calculated as percentages of the pre-slaughter live body weight of birds.

2.4 Blood chemistry

On day 35, birds were euthanized; blood samples were collected in vacuum blood collection tubes and centrifuged at $3000 \times g$ for 15 min to obtain serum; the samples were then stored frozen at −20 °C until analyzed. Total protein ($g\,dl^{-1}$, Ref.: 1085; wavelength 545 nm), albumin ($g\,dl^{-1}$, Ref.: 1007; wavelength 630 nm), total cholesterol ($mg\,dl^{-1}$, Ref.: 1082; wavelength 500 nm), triglycerides ($mg\,dl^{-1}$, Ref.: 87; wavelength 505 nm), and HDL ($mg\,dl^{-1}$, Ref.: 13; wavelength 500 nm) were measured using an automated chemistry analyzer (Mindray Bio-Medical Electronics Co., Shenzhen, China). By deducting serum albumin from serum total protein, serum globulin was computed. Meanwhile, blood glucose was measured in fresh blood collected during the procedure using a home glucose monitor (Accu-Chek Active, Roche Diagnostics, Basel, Switzerland).

2.5 Determination of antioxidant profile

Breast muscle samples were homogenized in an ice-cold homogenization buffer (e.g., 50 mM phosphate buffer, pH 7.0, containing 5 mM EDTA and 1 mM 2-mercaptoethanol). After centrifugation at 4000 rpm for 10–20 min at 2–8 °C, the supernatant was stored at −80 °C until analysis was performed. Using colorimetric method with commercial kits, the activity of total antioxidant capability (TOC catalog number ab65329; wavelength 570 nm), superoxide dismutase (SOD catalog number ab65354; wavelength 450 nm) and malondialdehyde (MDA catalog number ab118970; wavelength 570 nm) were assayed, glutathione peroxidase (GPx catalog number ab102530; wavelength 340 nm) by using the Ultraviolet (UV) method with commercial kits (Abcam Limited, Cambridge, UK) according to the manufacturer's instructions.

2.6 Determination of omega-3 and omega-6 PUFA in breast meat

Prior to analysis, breast tissue samples were thawed at 4 °C for 12 h and then homogenized using a tissue homogenizer (IKAT18 basic, Ultra-Turrax, Germany). The FA content (analyzed as FA methyl esters, FAMEs) of the analyzed samples was extracted, saponified, and methylated as described by Czauderna et al. (2007). After that, it was analyzed on a gas chromatograph (GC-2010, SHIMADZU, Tokyo, Japan) equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 m film thickness), a quadruple mass selective (MS) detector (Model GCMS-QP2010 Plus EI), and an injection port. Helium was the carrier gas, operated at a constant pressure and initial flow rate of $1\,mL\,min^{-1}$. FAME in examined samples was determined as described previously (Czauderna et al., 2007).

2.7 Statistical analysis

Statistical analysis was carried out using a general linear model (GLM) of the SAS (2013) program. The significant differences between treatment means were tested by the Duncan multiple range test (Steel et al., 1997). All percentages were subjected to arcsine transformation to approximate normal distribution before analysis. The data are presented as means and standard error of means (SEM). Probability values of less than 0.05 ($P < 0.05$) were significant. Two-way analysis of variance (ANOVA) was used to evaluate the effect of different treatments and their interactions on different measured parameters. Statistical analysis used the following model:

$$Y_{ijk} = \mu + D_i + T_j + D_i \times T_j + e_{ijk},$$

where Y_{ijk} = observed value; μ = the population mean; D_i = fixed effect of stocking densities (NSD and HSD); T_j = treatment effect (control, walnut oil, tea tree oil, and walnut oil + tea tree oil); $D_i \times T_j$ = the interaction between stocking densities and treatments; and e_{ijk} = experimental error.

3 Results

3.1 Growth performance

Growth performance results are shown in Table 2. It shows that the interaction between the stocking density and dietary treatments was significant for FBW, AWG, FI, and FCR ($P < 0.05$) but not for initial BW and mortality % ($P > 0.05$). The FBW and AWG were significantly lower in the HSD control group than in the NSD control group ($P < 0.05$). The FBW, AWG, and FI of control birds reared under NSD were significantly higher than those of the birds under HSD ($P < 0.05$). FCR was improved in birds reared under HSD compared to the birds reared under NSD ($P < 0.05$). Dietary supplementation with WO alone or in combination with TOL significantly increased these two parameters compared with the control and TOL groups ($P < 0.05$). Stocking density exerted a significant main effect on birds' performance. Birds reared under HSD had significantly lower FI ($P < 0.05$) and FCR ($P < 0.05$) across all treatments (0, WO, TOL, and combination) than birds reared

Table 2. Growth performance indices and mortality rate of broiler chicks of broilers fed diets supplemented with WO and/or TOL or without.

Treatments		Parameters					
		IBW (g bird^{-1})	FBW (g bird^{-1})	AWG (g bird^{-1})	FI (g bird^{-1})	FCR	Mortality (%)
Density effect							
	NSD (10 b m^{-2})	42.50	2400^{a}	2358^{a}	3572^{a}	1.52^{a}	3.33
	HSD (16 b m^{-2})	42.52	2335^{b}	2275^{b}	3229^{b}	1.42^{b}	4.17
SEM		0.04	17.03	19.22	19.78	0.01	1.11
P value		0.7970	0.0033	0.0026	< 0.0001	< 0.0001	0.6112
Herbal oils effect							
	0	42.51	2298^{b}	2255^{b}	3521^{a}	1.55^{a}	3.75
	WO	42.44	2463^{a}	2397^{a}	3333^{c}	1.39^{d}	3.75
	TOL	42.53	2259^{b}	2216^{b}	3352^{bc}	1.51^{b}	3.75
	Combination	42.56	2424^{a}	2359^{a}	3397^{b}	1.43^{c}	3.75
SEM		0.06	21.77	26.60	53.57	0.02	1.61
P value		0.4788	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1.000
Interaction effect							
NSD (10 b m^{-2})	0	42.53	2409^{a}	2367^{a}	3758^{a}	1.59^{a}	3.33
	WO	42.33	2468^{a}	2426^{a}	3518^{bc}	1.45^{c}	3.33
	TOL	42.57	2274^{b}	2232^{bc}	3472^{c}	1.56^{a}	3.33
	Combination	42.57	2450^{a}	2408^{a}	3541^{b}	1.47^{bc}	3.33
HSD (16 b m^{-2})	0	42.50	2228^{b}	2186^{c}	3284^{d}	1.50^{b}	4.17
	WO	42.50	2459^{a}	2379^{a}	3148^{e}	1.33^{e}	4.17
	TOL	42.50	2249^{b}	2207^{c}	3232^{d}	1.46^{bc}	4.17
	Combination	42.56	2407^{a}	2328^{ab}	3254^{d}	1.40^{d}	4.17
SEM		0.08	30.98	35.68	17.00	0.01	2.37
P value		0.5333	0.0219	< 0.0001	< 0.0001	< 0.0001	0.99

$^{a–e}$ Means within a row with no common superscripts are significantly different ($P < 0.05$). IBW, initial body weight; FBW, final body weight; AWG, average weight gain; FI, feed intake; FCR, feed conversion ratio; SEM, standard error of means; WO, walnut oil; TOL, tea tree oil; combination, walnut oil + tea tree oil. NSD, normal stocking density; HSD, high stocking density.

under NSD. Birds treated with WO showed a greater reduction in FCR than those treated with other additives under both NSD and HSD conditions. Birds fed WO as the main effect had significantly higher FBW and AWG, and higher FI than the control and TOL groups ($P < 0.05$). Moreover, the efficiency of reducing FCR was highest compared to when birds were fed WO alone ($P < 0.05$).

3.2 Carcass traits

The effects of dietary additives on carcass traits are presented in Table 3. There was a significant interaction between stocking density and dietary treatments for dressing percentage (DP), breast, and thigh meat percentages. Dietary supplementation with WO alone or in combination with TOL achieved the highest values of dressing, breast, and thigh in birds exposed to HSD or NSD in comparison with the other interactions. However, no interactions or main effects were observed for the other carcass cuts, including wings, neck, and organ weights (liver, heart, spleen, and gizzard), and abdominal fat percentage. Regarding the main effect of stocking density, dressing and breast percentages were significantly lower in the HSD group than in the NSD group ($P < 0.05$). However, dietary supplementation with WO alone or in combination with TOL significantly increased DP, breast, and thigh meat percentages compared with the control and TOL groups ($P < 0.05$).

3.3 Blood chemistry

The results of blood analysis are presented in Table 4. The results show a significant interaction between stocking density and treatments for glucose, cholesterol, triglycerides, HDL, and LDL ($P < 0.05$). When compared to the control, dietary supplementations with oils improved all lipid metabolites in birds reared under NSD or HSD. However, no interactions or main effects were observed for total protein, albumin, and globulin content ($P > 0.05$). Concentrations of glucose, cholesterol, triglycerides, HDL, and LDL did not differ between the NSD and HSD treatment groups. However, dietary

Table 3. Carcass characteristics of different experimental treatments of broilers fed diets supplemented with WO and/or TOL.

Treatments		DP	Carcass cuts (% of BW)				Organ weights (% of BW)				Abdominal fat
		(%.)	Breast	Thigh	Wings	Neck	Liver	Heart	Spleen	Gizzard	(% of BW)
Density effect											
	NSD ($10\,b\,m^{-2}$)	77.64[a]	33.37[a]	28.15	7.31	4.26	1.96	0.50	0.21	1.13	1.22
	HSD ($16\,b\,m^{-2}$)	74.74[b]	31.67[b]	26.83	7.35	4.26	1.96	0.48	0.21	1.14	1.22
SEM		1.08	0.64	0.57	0.04	0.04	0.04	0.01	0.01	0.03	0.05
P value		0.0048	0.0109	0.0531	0.5664	0.9240	0.9379	0.4217	0.7585	0.8088	0.9984
Herbal oils effect											
	0	74.45[b]	31.48[b]	26.57[b]	7.41	4.31	1.95	0.49	0.20	1.14	1.23
	WO	79.40[a]	34.30[a]	29.04[a]	7.31	4.23	1.98	0.49	0.22	1.13	1.21
	TOL	72.13[b]	30.30[b]	25.78[b]	7.29	4.23	2.00	0.48	0.20	1.13	1.21
	Combination	78.77[a]	33.98[a]	28.58[a]	7.29	4.27	1.92	0.50	0.22	1.13	1.24
SEM		0.84	0.61	0.61	0.05	0.06	0.05	0.02	0.01	0.05	0.07
P value		< 0.0001	0.0003	0.0048	0.4819	0.8316	0.8604	0.8492	0.0954	0.9984	0.9874
Interaction effect											
NSD ($10\,b\,m^{-2}$)	0	78.97[a]	33.90[a]	28.96[a]	7.34	4.27	1.94	0.49	0.20	1.11	1.24
	WO	79.77[a]	34.94[a]	28.85[a]	7.31	4.23	1.98	0.5	0.23	1.13	1.19
	TOL	72.31[b]	30.19[b]	26.11[ab]	7.27	4.25	1.99	0.49	0.20	1.13	1.21
	Combination	79.51[a]	34.43[a]	28.68[a]	7.31	4.28	1.94	0.51	0.22	1.13	1.25
HSD ($16\,b\,m^{-2}$)	0	69.93[c]	29.06[b]	24.17[b]	7.49	4.35	1.96	0.49	0.19	1.17	1.22
	WO	79.04[a]	33.65[a]	29.23[a]	7.32	4.24	1.99	0.48	0.21	1.13	1.22
	TOL	71.95[b]	30.41[b]	25.45[b]	7.31	4.22	2.00	0.47	0.21	1.14	1.21
	Combination	78.03[a]	33.54[a]	28.48[a]	7.27	4.26	1.91	0.50	0.22	1.12	1.24
SEM		0.54	0.59	0.54	0.08	0.19	0.08	0.03	0.02	0.07	0.10
P value		< 0.0001	< 0.0001	0.0003	0.78	0.99	0.99	0.98	0.29	0.99	1.00

[a, b] Means within a row with no common superscripts are significantly different ($P < 0.05$). SEM, standard error of means. HDL, high-density lipoprotein; LDL, low-density lipoprotein; WO, walnut oil; TOL, tea tree oil; combination, walnut oil + tea tree oil; NSD, normal stocking density; HSD, high stocking density; BW, body weight.

additives, either WO or TOL, individually or in combination, significantly reduced glucose, cholesterol, triglycerides, and LDL content, and increased HDL content compared to the control ($P < 0.05$), regardless of HSD.

3.4 Antioxidant profile of breast meat

The antioxidant profile of breast tissue is presented in Table 5. A significant interaction ($P < 0.05$) between density and dietary treatments was observed for total antioxidant capacity (TOC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) concentrations. Supplementation of WO and TOL individually and in combination significantly increased TOC, SOD, and GPx concentrations, and decreased MDA compared to the control, regardless of HSD. When compared to control, dietary supplementations with oils improved antioxidant indices in birds reared under NSD or HSD. The concentrations of SOD and GPx in the HSD groups were significantly lower than in the NSD groups ($P < 0.05$).

3.5 Fatty acid composition of breast meat

Table 6 shows the fatty acid content of breast meat tissue. Interactions affect omega-3 fatty acid content and the omega-3 : omega-6 fatty acid ratio ($P < 0.05$) but not omega-6 fatty acid content ($P > 0.05$). Supplementation with WO, TOL, or their combination improved the omega-3 content and the omega-3 : omega-6 in birds reared under HSD. Stocking density did not affect omega-3, omega-6 fatty acids, or the omega-3 : omega-6 ratio. However, among the dietary treatments, supplementation with WO either alone or in combination with TOL resulted in higher omega-3 fatty acid content in breast meat compared to TOL alone. The omega-6 : omega-3 ratio was significantly higher in the control group than in the dietary additive-treated groups.

4 Discussion

This study investigates the effects of WO and TOL on growth performance, carcass traits, antioxidant profile, fatty acid composition, and lipid profile of broilers reared with HSD. Results showed that birds reared under HSD had reduced performance, antioxidant capacity, and lipid profile, as well as impaired meat yield and quality. Supplementation with WO and TOL, individually, increased FBW, AWG, and FI, and reduced FCR. Supplementation with WO alone was more effective than TOL in improving performance parameters. Both WO and TOL increased DP and the percentages of breast and thigh meat, while reducing glucose, triglycerides, and LDL, and increasing HDL. In addition, both oils en-

Table 4. Serum biochemical indices of birds at different experimental treatments of broilers fed diets supplemented with WO and/or TOL or without.

Treatments		Parameters							
		Glucose ($mg\,dl^{-1}$)	Total protein ($g\,dl^{-1}$)	Albumin ($g\,dl^{-1}$)	Globulin ($g\,dl^{-1}$)	Cholest. ($mg\,dl^{-1}$)	Triglyc. ($mg\,dl^{-1}$)	HDL ($mg\,dl^{-1}$)	LDL ($mg\,dl^{-1}$)
Density effect									
	NSD ($10\,b\,m^{-2}$)	119.78	3.35	1.83	1.53	127.80	138.88	61.88	38.15
	HSD ($16\,b\,m^{-2}$)	119.11	3.27	1.67	1.60	130.74	139.08	61.79	41.13
SEM		3.04	0.05	0.06	0.06	5.59	4.17	3.30	7.92
P value		0.8206	0.2551	0.0913	0.3972	0.3519	0.9621	0.9756	0.4996
Herbal oils effect									
	0	132.97^{a}	3.35	1.74	1.62	159.17^{a}	156.2^{a}	45.83^{b}	82.08^{a}
	WO	118.00^{b}	3.36	1.83	1.54	119.12^{b}	133.7^{b}	67.25^{a}	$25.12^{\mathbf{b}}$
	TOL	112.53^{b}	3.24	1.73	1.52	118.45^{b}	130.5^{b}	67.50^{a}	$24.85^{\mathbf{b}}$
	Combination	114.28^{b}	3.29	1.72	1.58	120.36^{b}	135.4^{b}	66.75^{a}	$26.53^{\mathbf{b}}$
SEM		2.75	0.07	0.09	0.08	3.01	4.00	2.58	4.13
P value		0.0003	0.6277	0.8114	0.8625	< 0.0001	0.0019	< 0.0001	< 0.0001
Interaction effect									
NSD ($10\,b\,m^{-2}$)	0	136.61^{a}	3.47	1.92	1.55	157.67^{a}	159.5^{a}	46.33^{b}	79.43^{a}
	WO	119.00^{b}	3.36	1.85	1.51	116.88^{b}	133.0^{b}	65.67^{a}	24.62^{b}
	TOL	110.00^{b}	3.29	1.78	1.52	118.33^{b}	129.5^{b}	68.00^{a}	24.43^{b}
	Combination	113.50^{b}	3.31	1.78	1.53	118.33^{b}	133.5^{b}	67.50^{a}	24.13^{b}
HSD ($16\,b\,m^{-2}$)	0	129.33^{a}	3.23	1.55	1.68	160.67^{a}	153.0^{a}	45.33^{b}	84.73^{a}
	WO	117.00^{b}	3.37	1.81	1.56	121.35^{b}	134.5^{b}	68.83^{a}	25.62^{b}
	TOL	115.00^{b}	3.20	1.67	1.53	118.57^{b}	131.5^{b}	67.00^{a}	25.27^{b}
	Combination	115.05^{b}	3.28	1.65	1.63	122.38^{b}	137.3^{b}	66.00^{a}	28.92^{b}
SEM		3.60	0.15	0.11	0.17	2.84	3.51	2.33	3.94
P value		< 0.001	0.960	0.535	0.998	< 0.0001	0.001	< 0.0001	0.001

[a, b] Means within a row with no common superscripts are significantly different ($P < 0.05$). SEM, standard error of means. HDL, high-density lipoprotein; LDL, low-density lipoprotein; WO, walnut oil; TOL, tea tree oil; combination, walnut oil + tea tree oil; cholest, cholesterol; triglyc. triglycerides; NSD, normal stocking density; HSD, high stocking density.

hanced antioxidant capacity by increasing TOC, SOD, and GPx, and by reducing MDA. They also enriched the meat with higher levels of omega-3 fatty acids, resulting in a lower omega-6 : omega-3 fatty acid ratio. However, combining these oils improved the parameters mentioned above but not to a greater extent than individual supplementation with WO alone.

Growth performance is a key indicator to analyze the efficacy of supplements in the poultry industry. High stocking density negatively affects performance by reducing FI, FBW, and meat yield (Thema et al., 2022). Consistent with the previous finding, birds reared under HSD in this study had reduced FI, AWG, and FBW, and increased FCR compared with those under NSD. Dietary supplementation with WO and TOL increased final BWG and FI, and reduced FCR in this study under both NSD and HSD conditions. In addition, both oils increased dressing percentage and breast and thigh meat yield. They also increased omega-3 fatty acids and the omega-6 : omega-3 fatty acid ratio.

The performance and carcass trait benefits of TOL supplementation align with previous research, which suggests that TOL supplementation improved performance and carcass traits under both normal and HSD conditions (Abo Ghanima et al., 2021; Liu et al., 2023; Qu et al., 2019). Dietary TOL reduces oxidative stress, apoptosis, and inflammation while improving tight junction proteins and villus structure (Liu et al., 2023; Wang et al., 2025). Moreover, TOL possesses antifungal, bacteriostatic, and germicidal activity against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and yeast, *Candida albicans* (Puvača et al., 2019), which may help to improve gut function without affecting disease. Dietary supplementation with TOL has also been reported to increase beneficial *Lactobacillus* in the chicken gut, further supporting its potential to increase helpful bacteria by killing the harmful organisms in the intestine (Qu et al., 2019). The primary bioactive compound of TOL, terpinen-4-ol, can effectively kill intestinal pathogenic bacteria (Cordeiro et al., 2020), further supporting TOL's antibacterial potential. These TOL functions help to explain improved gut health, which strengthens nutrient absorption, leading to more weight gain, improved FCR, and higher meat yield. The performance benefits are more variable with WO. Previous studies have reported that WO negatively affects final BW, but WO, walnut seed, and

Table 5. Antioxidant activity in breast meat tissue of birds at different experimental treatments of broilers fed diets supplemented with WO and/or TOL or without.

Treatments		Parameters			
		TOC ($U\,mg^{-1}$ protein)	SOD ($U\,mg^{-1}$ protein)	GPx ($U\,mg^{-1}$ protein)	MDA ($U\,mg^{-1}$ protein)
Density effect					
	NSD ($10\,b\,m^{-2}$)	2.20	39.71^{a}	19.11^{a}	1.89
	HSD ($16\,b\,m^{-2}$)	2.00	34.42^{b}	16.19^{b}	2.02
SEM		0.16	2.44	1.15	0.11
P value		0.0780	0.0020	0.0005	0.1253
Herbal oils effect					
	0	1.30^{b}	24.27^{b}	11.67^{b}	2.56^{a}
	WO	2.20^{a}	43.04^{a}	19.12^{a}	1.70^{b}
	TOL	2.35^{a}	39.57^{a}	20.56^{a}	1.76^{b}
	Combination	2.54^{a}	41.38^{a}	19.25^{a}	1.81^{b}
SEM		0.11	1.82	0.93	0.07
P value		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Interaction effect					
NSD ($10\,b\,m^{-2}$)	Control	1.61^{b}	28.41^{b}	13.68^{c}	2.31^{a}
	WO	2.30^{a}	45.16^{a}	20.35^{ab}	1.69^{c}
	TOL	2.38^{a}	40.91^{a}	22.18^{a}	1.70^{c}
	Combination	2.52^{a}	44.34^{a}	20.24^{ab}	1.86^{c}
HSD ($16\,b\,m^{-2}$)	Control	0.99^{c}	20.14^{c}	9.65^{d}	2.81^{b}
	WO	2.10^{ab}	40.91^{a}	17.90^{b}	1.72^{c}
	TOL	2.32^{a}	38.23^{a}	18.94^{ab}	1.81^{c}
	Combination	2.57^{a}	38.42^{a}	18.27^{b}	1.75^{c}
SEM		0.16	2.32	2.04	0.24
P value		0.0001	< 0.0001	< 0.0001	< 0.0001

[a–d] Means within a row with no common superscripts are significantly different ($P < 0.05$). SEM, standard error of means. WO, walnut oil; TOL, tea tree oil; combination, walnut oil + tea tree oil; TOC, total antioxidant capacity; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde.

walnut meal enhance omega-3 fatty acid content and the omega-6: omega-3 ratio, and modulate the intestinal microbiota (Adeyemi et al., 2020; Jiang et al., 2024; Untea et al., 2025). Conversely, WO cake increases performance, DP, and breast and thigh meat yield when used as a 20 % substitute for soybean meal in boilers, and enhances villus structures (Beigh et al., 2025). The WO oil is rich in omega-3 fatty acids, which have antioxidant and anti-inflammatory properties (Fan et al., 2023). Increased omega-3 fatty acids reduce intestinal inflammation and ROS production, which support gut structure and function (Calder, 2015; Durkin et al., 2021; Sun et al., 2025). Healthy birds allocate more nutrients to muscle growth than to fat deposition, indicating efficient digestive physiology and metabolic function to gain more body weight. The improved performance observed in this study with WO may be due to improvements in intestinal structure and overall gut health resulting from reduced inflammation and oxidative damage, thereby increasing nutrient availability for absorption and subsequent muscle development. Moreover, in this study, both oils reduced oxidative stress and improved lipid profile, suggesting enhanced utilization of dietary fat for energy.

Stress conditions promote the production of excessive reactive oxygen species and reduce antioxidant enzyme activity, thereby diminishing the birds' ability to scavenge free radicals. As a result, birds' performance and meat quality are impaired under stress conditions (Miao et al., 2020; Oke et al., 2024). In this study, birds exposed to HSD showed reduced SOD and GPx activity compared with those under NSD conditions, confirming the sensitivity of the antioxidant system to stocking-density-induced stress. Interestingly, dietary supplementation with TOL and WO increased TOC, SOD, and GPx while reducing the MDA in this study, consistent with observations reported in broilers, pigs, and in vitro systems (Abo Ghanima et al., 2021; Demirel et al., 2024; Qu et al., 2019; Zhang et al., 2021). The improved oxidative stability associated with TOL may be attributed to its major components, such as α-terpinene, α-terpinolene, and γ-terpinene, which have strong hydrogen-donating and radical-quenching properties (Kim et al., 2004). Mechanis-

Table 6. Contents of omega-3 and omega-6 in breast meat tissue of birds under different experimental treatments of broilers fed diets supplemented with WO and/or TOL or without.

Treatments		Parameters		
		Omega-3	Omega-6	Omega-3 : Omega-6
Density effect				
NSD ($10\,b\,m^{-2}$)		1.68	7.04	4.90
HSD ($16\,b\,m^{-2}$)		1.61	6.95	5.17
SEM		0.18	0.27	0.71
P value		0.1487	0.8157	0.5402
Herbal oils effect				
	0	0.76^{c}	6.40	8.67^{a}
	WO	2.18^{a}	7.28	3.33^{c}
	TOL	1.49^{b}	6.91	4.68^{b}
	Combination	2.15^{a}	7.39	3.46^{bc}
SEM		0.05	0.35	0.36
P value		< 0.0001	0.2710	< 0.0001
Interaction effect				
NSD ($10\,b\,m^{-2}$)	0	0.80^{c}	6.53	8.37^{a}
	WO	2.22^{a}	7.12	3.20^{b}
	TOL	1.54^{b}	6.95	4.52^{b}
	Combination	2.16^{a}	7.56	3.51^{b}
HSD ($16\,b\,m^{-2}$)	0	0.71^{c}	6.28	8.97^{a}
	WO	2.15^{a}	7.43	3.46^{b}
	TOL	1.44^{b}	6.87	4.85^{b}
	Combination	2.13^{a}	7.23	3.40^{b}
SEM		0.07	0.55	0.61
P value		< 0.0001	0.7620	< 0.0001

$^{a-c}$ Means within a row with no common superscripts are significantly different ($P < 0.05$). SEM, standard error of means; WO, walnut oil; TOL, tea tree oil; combination, walnut oil + tea tree oil.

tically, TOL has been shown to stimulate the nuclear factor erythroid-2-related factor 2 signaling pathway in fish, which upregulates antioxidant-related genes (Liu et al., 2022b). Similarly, the observed increase in antioxidant status with WO can be explained by the naturally high concentrations of tocopherols and phenolic compounds, both of which exhibit strong free-radical-scavenging activity, interrupt lipid peroxidation chain reactions, and chelate pro-oxidant metal ions, thereby maintaining cellular redox balance (Gao et al., 2022a). In summary, these findings highlight the potential of TOL and WO to enhance oxidative defense capacity, thereby counteracting stress-induced impairments in broilers.

Effective lipid metabolism is essential in broilers, as lipids provide energy for growth and organ development, and contribute to overall health and productivity (Cherian, 2015). In the present study, both TOL and WO reduced serum glucose, cholesterol, triglycerides, and LDL, while increasing HDL in broilers, compared to control birds; this aligns with previous studies that used TOL, WO, and other essential oils in broilers and humans (Abo Ghanima et al., 2021; Puvača et al., 2022; Zibaeenezhad et al., 2017). Supplementation with TOL can reduce lipids by inhibiting the liver and hepatopancreas from producing excess fat and by helping the body to use stored fat for energy, thereby lowering triglycerides, cholesterol, and LDL, and preventing fat accumulation in the liver (Liu et al., 2022a). In addition, the terpenes present in TOL interact with insulin signaling pathways, increase glucose uptake, and therefore reduce blood glucose levels, consistent with our findings (Papada, 2025).

Dietary WO has been reported to downregulate cholesterol-synthesis genes, including 3-hydroxy-3-methylglutaryl-CoA reductase, sterol regulatory element-binding protein 2, and cytochrome P450, and to increase cholesterol efflux by upregulating ATP-binding cassette subfamily G member 1 in vitro; consequently, it significantly reduced total cholesterol and triacylglycerol (Gao et al., 2022b). These findings indicate that both TOL and WO can modulate lipid metabolism, thereby supporting increased energy utilization for growth and organ development.

5 Conclusion

In conclusion, supplementation with WO and TOL, individually and in combination, improved performance, carcass traits, antioxidant activity, lipid profile, and fatty acid composition in broilers under HSD. However, the combination did not exhibit synergistic effects beyond those of WO alone. Overall, these findings indicate that TOL and WO supplementation can be effective strategies to improve performance and carcass yield (DP, breast, and thigh meat yield), meat quality (increase omega-3 fatty acids in meat), enhance antioxidant capacity, and improve the lipid profile. Further research on gut health, particularly the effects on the intestinal microbiota and mucosal integrity, is warranted to fully understand and confirm their potential benefits.

Data availability. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Competing interests. The contact author has declared that none of the authors has any competing interests.

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Ethical statement. Procedures of the experiment were approved by the Ethical Committee of the Faculty of Agriculture, Assiut University, Egypt (no. 2023/10/01) regarding the protection of animals used for scientific purposes.

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