META-ANALYSIS OF DIETARY ESSENTIAL OILS IN BROILER NUTRITION: GROWTH PERFORMANCE, ABDOMINAL FAT AND BLOOD LIPID CHARACTERISTICS

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Abstract. The use of essential oils (EO) as growth promoters in modern animal production is on the rise. Studies assessing the impact of EO on growth performance and health indices, however, have produced variable results. To assess the impact of EO on feed intake (FI), feed conversion ratio (FCR), body weight gain (BWG), abdominal fat, blood levels of triglycerides, total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in broilers, meta-analysis approach was used. A search performed on five online databases (Google Scholar, PubMed, ScienceDirect, Scopus, and Web of Science databases) yielded 1252 studies, of which 36 were eligible for meta-analysis. Data were aggregated using Der-Simonian and Laird's random-effects model, and results were presented as mean differences (MD) at 95% confidence intervals (CI). All the statistical analyses were done in OpenMEE software. Results indicated that EO had a beneficial effect on FI (MD = 0.75 g/bird; 0.04, 1.45; $P = 0.038$), BWG (MD = 2.10 g/bird; 1.36, 2.84; $P < 0.00$) and FCR (MD = –0.06; –0.08, –0.04; $P < 0.001$) in broilers. In comparison with the control, dietary EO supplementation improved abdominal fat content (MD = –0.21 g; –0.28, –0.13; $P < 0.001$), blood TC (MD = –3.85 mg/dl; –5.40, –2.30; $P < 0.001$), HDL (MD = 5.70 mg/dl; 1.48, 9.92; $P = 0.008$), and LDL (MD = –7.38 mg/dl; –11.69, –3.07; $P < 0.001$) in broilers. However, EO supplementation did not affect blood TG (MD = –0.06; –0.08, –0.04; $P < 0.001$) levels in broilers. Subgroup analyses revealed that dosage, type of EO, broiler strains, and rearing phase had positive effects on measured outcomes. Meta-regression indicates that the type of EO used was a significant predictor of FI, FCR, BWG, abdominal fat, and blood TC in broilers fed EO-supplemented diets. Likewise, dosage was a significant predictor of FCR and BWG in broilers fed EO-supplemented diets. These results suggest that EO can be added to chicken diets to improve the growth performance and health status of broilers. The present meta-analysis sets guidelines for standardized experimental designs on the use of EO in broiler feeding trials in the future.
Keywords: *feed intake, feed conversion, weight gain, cholesterol, triglycerides, subgroup analysis*

Introduction

According to Statista (2023), global meat production is projected to increase from 317 to 351 million metric tons between 2016 and 2024. This pattern is expected to continue as the world's population grows steadily. Thus, for chickens and livestock to provide safe and high-quality products that meet the needs of growing world population, feed conversion ratio (FCR), calculated as the amount of feed consumed per unit of live weight gain, needs to be improved. In broiler production, feed accounts for 60-70% of the total cost of production (Thirumalaisamy, 2019). From this point of view, the use of essential oils (EO) in broiler nutrition to enhance digestion, nutrient utilization, and growth has received increased attention in recent times (Su et al., 2021; Puvaca et al., 2022; Caroprese et al., 2023). Essential oils are highly concentrated aromatic liquids extracted from different parts of plants, including seeds, bark, fruits, flowers, and roots (Huang et al., 2024). Essential oils are also known as secondary metabolites because they are

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produced from primary metabolites during plant metabolism (Dehariya et al., 2020). The composition and amount of EO in the same plant can differ depending on the part of the plant used (Puvaca et al., 2022). Studies have shown that EO has a wide variety of important biological effects in non-ruminants, including appetite and digestive enzyme stimulant, antioxidant, antifungal, and antimicrobial properties (Mangalagiri et al., 2021). Dietary EO supplementation also influences the gut by stimulating the release of endogenous digestive secretion (bile, mucus, saliva, and pancreatic amylase), resulting in increased feed efficiency and nutrient uptake (Petrolli et al., 2012). Essential oils are safe as feed additives in human and animal nutrition (FDA, 2004).

Studies have shown that EO is rich in important bioactive compounds such as phenols, polyphenols, and terpenoids, which, when added to the chicken diet, can alter gut microbiota composition in favor of the growth of beneficial microbes (Mangalagiri et al., 2021). Such EO-induced changes in gut microbiota composition can optimize feed digestibility and nutrient utilization, leading to improvements in growth performance in broiler chickens (Stefanello et al., 2019; Su et al., 2021). These findings are consistent with the results of Herve et al. (2019), who reported enhanced growth performance in broiler chicken fed a basal diet supplemented with ginger EO at 50, 100, and 150 mg/kg. Nehme et al. (2021) discovered that EO supplementation improves growth performance and reduces blood and meat cholesterol levels, as well as abdominal fat, by hindering the activities of several enzymes connected to cholesterol and fat synthesis. In a more recent study, Huang et al. (2024) found increased growth dynamics in broiler chickens fed diets supplemented with essential oil components (thymol and carvacrol) at 200 mg/kg and decreased FI when supplemented at 400, 600, and 800 mg/kg. When broiler chickens were offered diets supplemented with peppermint EO at an inclusion level of 150 mg/kg, the FI and BWG decreased, while FCR increased when compared to the control (Ghazanfari et al., 2024). In the Arbor Acres broiler strain, Yang et al. (2019) found that supplementation of cinnamon EO at 100 and 200 mg/kg diet resulted in a decrease in FI and BWG and increased FI and BWG at 400 and 800 mg/kg. The varied responses among broiler chickens offered an EO-supplemented diet reflect differences in diet composition, chemical composition of EO, broiler strain used, dosage, and type of EO.

The use of meta-analysis, a statistical method that combines multiple published studies on the same topic in animal agriculture to increase statistical power, resolve uncertainty, identify knowledge gaps, and develop new insights, has been evaluated (Irawan et al., 2021; Adli et al., 2023; Ogbuewu et al., 2024). However, there is a scanty study on the meta-analysis of EO supplements on broiler performance. Therefore, this meta-analysis reports the impact of EO on growth dynamics, abdominal fat, and blood lipid profiles of broilers. In addition, factors that led to the inconsistent results in trials that assessed the effect of EO supplements on broiler performance will be determined using the meta-regression method. Data to be obtained will help broiler farmers, policymakers, and other stakeholders to make informed decisions on the effectiveness of EO supplements in improving broiler productivity and health.

Materials and methods

Article identification and selection criteria

The articles used for this study were chosen based on the following criteria: (i) full-text studies reporting the impact of diets with and without EO supplementation on at least

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one of the outcomes of interest (FI, BWG, FCR, abdominal fat, blood TG, TC, HDL, and LDL) in broilers; (ii) feed and water were free from antibiotics and other growth promoters; and (iii) study was published in peer-reviewed journals. One thousand two hundred and fifty-two (1252) articles were identified following a systematic search performed on Google Scholar, PubMed, ScienceDirect, Scopus, and Web of Science databases. The search was executed using the keywords: "essential oils," "broiler chicken," "broilers," "blood lipids," "abdominal fat," and "growth performance." PICO (Population, Intervention, Comparators, Outcomes) format was adopted, where P = broilers, I = EO supplementation, C = diet without EO supplementation, and O = measured outcomes. Thirty-six studies met the eligibility criteria following the steps described in *Figure 1* and were used for the study. Articles were selected based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines of Page et al. (2021).

Database development

Data screening and extraction were done by the three authors, and disagreement on whether to include an article or not was resolved by consensus. Studies that met the eligibility criteria were downloaded into Zotero software, a free and open-source reference management software. Data was extracted on study characteristics (author's name, publication year, study country, and continent), study-level characteristics or covariates (i.e., rearing phase, broiler strain, dosage, and type of EO), and number of broilers in the control and treatment groups from each study that met the eligibility criteria. Rearing phases were the starter phase (d 1-21), finisher phase (d 22 - 49), and overall phase (d 1 - 49). Dose level was categorized as 1 - 50, 51-100, 101-200, 201-300, 301-400, 401-500, 501-600 and > 600 mg EO/kg. Broiler strains used were Ross, Cobb, Arbor Acres, Lohmann, and Hybro, whereas the EO types were cinnamon, oregano, thyme, ginger, garlic, turmeric, rosemary, liquidambar, star anise, satureja, peppermint EO, and aspects of their blends. This sub-division was based on the data provided by the authors whose studies were used for meta-analysis. Garlic and turmeric EO were not used in subgroup analysis due to the low sample size. Data presented as figures were digitalized using WebplotDigitizer (Rohatgi, 2022). The characteristics of the 36 studies used for the meta-analysis are presented in *Table 1*.

Statistical analysis

Data were analyzed using OpenMEE software (Wallace et al., 2016) and the R Core Team (2020) Data were pooled using a random-effects model following the method of Der-Simonian and Laird (1986). Effect sizes were presented as mean difference (MD) at 95% confidence intervals. Egger et al. (1997) method was used to assess the existence of publication bias, which is the propensity for research with positive or statistically significant outcomes to be published more often than studies with null or negative outcomes. Heterogeneity (I^2), which measures variability in the outcomes of studies included in the meta-analysis, was assessed using Chi-squared-statistics. The association between moderators (broiler strain, supplementation level, and type of EO) and measured outcomes was explored using meta-regression and subgroup analyses. Meta-regression analysis was not performed on outcomes with fewer than 10 studies owing to low statistical power (Borenstein et al., 2009). In the present study, subgroup analysis was not conducted on strata with <3 comparisons because of the low sample

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size (Ogbuewu et al., 2022a). Results were deemed significant when the probability value was less than 5%.

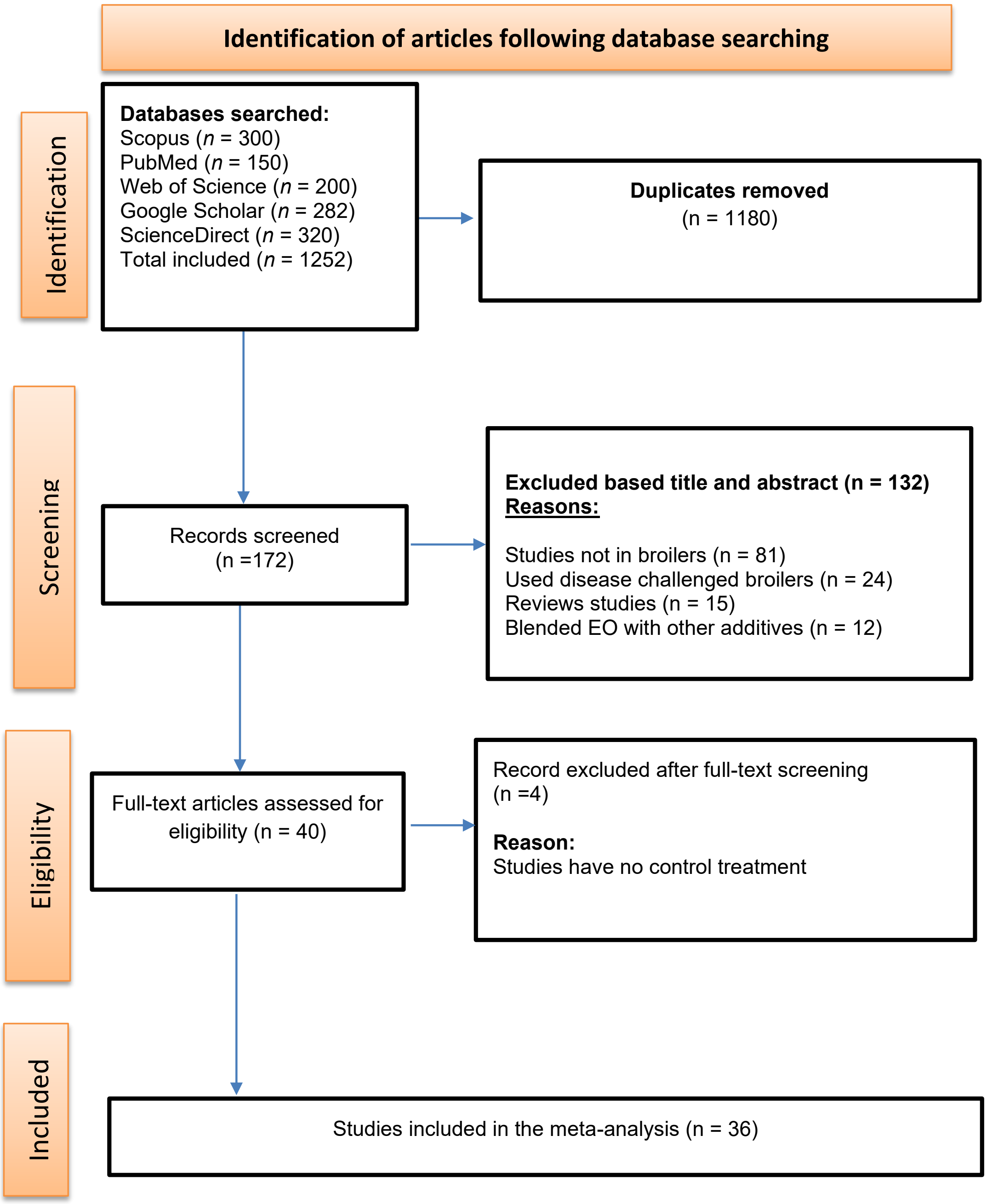

Figure 1. *Study selection flow chart for the meta-analysis*

Results

Article characteristics

Table 1 presents the features of the 36 studies that were used for meta-analysis. The included articles were published for 16 years (2008-2024). The included studies were conducted in 19 countries spanning five study continents. Most of the studies used the Ross strain and were conducted in Europe.

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Table 1. *Characteristics of included studies.*

Author	Country	Continent	Covariates				Outcomes
			BS	Rearing phase	Type of EO	Dosage	
Loh et al. (2008)	Malaysia	Asia	Ross	1.0-21	Blended EO	0-150	1,2,3
Park and Kim (2008)	Korea	Asia	Ross	1.0-35	Blended EO	0-200	1,2,3,4
Ciftci et al. (2009)	Turkey	Europe	Ross	5.0-35	Cinnamon EO	0-1000	1,2,3,4
Isabel and Santos (2009)	Spain	Europe	Ross	1.0-46	Blended EO	0-100	2,3,4
Ciftci et al. (2010)	Turkey	Europe	Ross	1.0-35	Cinnamon EO	0-1000	6
Malayoglu et al. (2010)	Turkey	Europe	Ross	0-21	OEO	0-500	1,2,3,5,6,7
Tiihonen et al. (2010)	UK	Europe	Ross	0-21, 0-42	Blended EO	0-23	1,2,3
Amad et al. (2011)	Yemen	Asia	Cobb	1.0-21, 22-35, 1-35	Blended EO	0-1500	1,2,3
Amerah et al. (2011)	UK	Europe	Ross	1.0-21, 22-35, 1-35	Blended EO	0-100	1,2,3
Alp et al. (2012)	Turkey	Europe	Ross	1.0-21, 22-42, 1-42	OEO	0-300	1,2,3
Mueller et al. (2012)	Germany	Europe	Ross	1.0-35	TEO, OEO, TuEO, REO	0-150	1,2,3
Hashemipour et al. (2013)	Netherland	Europe	Ross	0-42	Blended EO	0-200	1,2,3
Betancourt et al. (2014)	Colombia	SA	Hybro	1.0-42	OEO	0-200	1,2,3
Humer et al. (2014)	Austria	Europe	Ross	1.0-35	Blended EO	0-1500	1,2,3,4
Saleh et al. (2014)	Egypt	Africa	Ross	1.0-42	TEO, GEO	0-300	1,2,3,5,6,7,8
Symeon et al. (2014)	Greece	Europe	Cobb	1.0-49	Cinnamon EO	nr	1,2,3,4
Pirgozliev et al. (2015)	UK	Europe	Ross	0-21	Blended EO	0-110	1,2,3
Basmacioglu-Malayoglu et al. (2016)	Turkey	Europe	Ross	1.0-21, 22-42, 1-42	Blended EO	0-300	1,2,3,4
Hafeez et al. (2016)	Greece	Europe	Cobb	1.0-42	Blended EO	0-150	1,2,3
Altop et al. (2017)	Turkey	Europe	Ross	1.0-21, 22-42, 1-42	Liquidambar EO	0-166.2	1,2,3,4,5,6,7,8
Ding et al. (2017)	China	Asia	AA	1.0-21, 22-42, 1-42	Star anise EO	0-200	1,2,3
Masouri et al. (2017)	Iran	Asia	Ross	1.0-21, 22-42, 1-42	Satureja EO	0-500	1,2,3,5,6,7,8
Mohiti-Asli and Ghanaatparast-Rashti (2017)	Iran	Asia	Ross	1.0-21, 22-42, 1-42	OEO	0-500	1,2,3
Paraskeuas et al. (2017)	Greece	Europe	Cobb	1.0-42	Blended EO	0-150	1,2,3, 5, 6
Hosseini and Meimandipour (2018)	Iran	Asia	Ross	1.0-21, 1-42	TEO	0-40	1,2,3,5,6,7,8
Reis et al. (2018)	Brazil	NA	Cobb	1.0-42	Blended EO	0-10000	6
Abdel-Wareth et al. (2019)	Egypt	Africa	Ross	1.0-21, 1-35	Peppermint EO	0-78	1,2,3,4
Attia et al. (2019)	Egypt	Africa	AA	1.0-36	Blended EO	0-150	1,2,3,4,5,6
Placha et al. (2019)	SR	Europe	Ross	1.0-28	TEO	0-1000	1,2,3
Yang et al. (2019)	China	Asia	AA	1.0-21	Cinnamon EO	0-800	1,2,3
Iqbal et al. (2021)	Pakistan	Asia	Ross	1.0-35	Blended EO	0-100	1,2,3,6,7,8
Elbaz et al. (2022)	Egypt	Africa	Ross	1.0-35	GrEO, LEO	0-100	1,2,3,4,5,6,7,8
Islam et al. (2023)	Pakistan	Asia	Ross	1.0-21	Blended EO	0-150	1,2,3
Ghazanfari et al. (2024)	Iran	Asia	Ross	1.0-42	Peppermint EO	0-150	1,2,3,4
Huang et al. (2024)	China	Asia	AA	1.0-21, 22-42, 1-42	Blended EO	0-800	1,2,3,4
Merdana et al. (2024)	Indonesia	Asia	Lohmann	1.0-33	Blended EO	0-200	1,2,3

nr not reported; *SA* South America; *SR* Slovak Republic; *NA* North America; *AA* Arbor Acres; *OEO* oregano essential oil; *GrEO* garlic essential oil; *TuEO* turmeric essential oil; *BS* broiler strain; *LEO* lemon essential oil; *GEO* ginger essential oil; *TEO* thyme essential oil; *REO* rosemary essential oil; 1- FI; 2-FCR; 3-BWG; 4 – abdominal fat; 5- TG; 6 – TC; 7 – HDL; 8 – LDL

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Pooled analysis

Results as presented in *Table 2* suggested that FI (MD = 0.75 g/bird; 0.04, 1.45; *P* = 0.038) and BWG (MD = 2.10 g/bird; 1.36, 2.84; *P* < 0.00) were increased in broilers offered EO supplements when compared to the controls. Broilers fed EO-based diets had improved FCR (MD = –0.06; –0.08, –0.04; *P* < 0.001). The abdominal fat and blood lipids of broilers fed EO-supplemented diets are shown in *Table 3*. Abdominal fat was lower in broilers fed EO diets than those fed control diets (MD = –0.21 g; –0.28, –0.13; *P* < 0.001EO supplementation decreased TC (MD = –3.85 mg/dl; –5.40, –2.30; *P* < 0.001) and increased HDL (MD = 5.70 mg/dl; 1.48, 9.92; *P* < 0.001) in broilers. The results showed that EO decreased blood LDL (MD = –7.38 mg/dl; –11.69, –3.07; *P* < 0.001) in broilers. In contrast, TG (MD = −1.50 mg/dl; −5.81, 2.81; *P* = 0.496) was not affected by EO supplementation.

***Table 2.** Effect of EO supplementation on growth performance of broilers*

Parameters	n	MD	(95% CI)	*p*-value	Heterogeneity		Egger test
					I^2 (%)	*p*-value	
FI (g/d/bird)	109	0.75	0.04, 1.45	0.038	88	<0.001	0.168
FCR	123	-0.06	-0.08, -0.04	<0.001	94	<0.001	0.542
BWG (g/d/bird)	123	2.10	1.36, 2.84	<0.001	96	<0.001	0.221

MD mean difference; *FI* feed intake; *FCR* feed conversion ratio; *BWG* body weight gain; *n* number of comparisons; *p* probability value; I^2, Inconsistency Index

***Table 3.** Effect of EO supplementation on abdominal fat content and blood lipid profiles of broilers*

Parameters (mg/dl)	n	MD	(95% CI)	*p*-value	Heterogeneity		Egger test
					I^2 (%)	*p*-value	
Abdominal fat (g)	26	-0.21	-0.28, -0.13	<0.001	98	<0.001	0.243
TG	23	-1.50	-5.81, 2.81	0.496	99	<0.001	0.971
TC	29	-3.85	-5.40, -2.30	<0.001	97	<0.001	0.854
HDL	19	5.70	1.48, 9.92	0.008	99	<0.001	0.085
LDL	17	-7.38	-11.69, -3.07	<0.001	99	<0.001	0.154

TG triglycerides; *TC* total cholesterol; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein

Restricted subgroup analysis

Growth dynamics

The effect of covariates on FI in broilers is displayed in *Table 4*. EO supplementation at 1-50 and 51-100 mg/kg increased FI in broilers. Birds on peppermint, liquidambar, and star anise EO treatments consumed more feed than those that received oregano EO. Feed intake was higher in Cobb (MD = 2.64 g/bird; 0.18, 5.10; *P* = 0.035) than in Lohmann (MD = –3.90 g/bird; –6.81, –1.00; *P* = 0.008). Feed intake was not affected by the rearing phase. The effect of covariates on FCR in broilers is shown in *Table 5*. EO intervention at 1-50, 51-100, 201-300, 401-500, 501-600, and > 600 mg/kg improved FCR. However, broilers fed diets with EO at 101-200 and 301-400 mg/kg and without EO had similar FCR. Broilers fed peppermint, oregano, satureja, and blended EO had statistically better FCR than those fed star anise EO. In contrast, FCR was not affected in broilers fed thyme,

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ginger, and cinnamon EO. Arbor Acres, Ross, Lohmann, and Cobb broilers offered EO-supplemented diets recorded better FCR than those offered control diets. Hybro strains fed EO-based diets had similar FCR to the control. However, FCR was not affected by the rearing phase. *Table 6* shows the impact of covariates on broiler BWG. BWG was affected by EO dosage, with broilers fed 1-50, 51-100, 101-200, 201-300, 301-400, 401-500, and > 600 mg EO/kg having higher BWG than the controls. Results show that BWG was affected in broilers fed 501-600 mg EO/kg. Higher BWG was found in broilers offered peppermint, thyme, liquidambar, satureja, star anise, and blended EO. BWG in broilers fed EO-supplemented diets was affected by the rearing phase.

Table 4. *Effect of covariates on FI in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	13	1.56	0.47, 2.65	0.005	48	0.028
	51-100	22	1.45	0.38; 2.52	0.008	64	<0.001
	101-200	30	0.64	-0.25; 1.53	0.159	73	<0.001
	201-300	16	-0.96	-3.26; 1.33	0.411	93	<0.001
	301-400	5	0.05	-1.00; 1.10	0.923	0	0.851
	401-500	12	2.48	-1.81; 6.76	0.257	98	<0.001
	501-600	4	-1.44	-4.60; 1.72	0.371	74	0.023
	> 600	7	-0.10	-1.22; 1.02	0.867	17	0.297
Type of EO	Blended EO	49	0.19	-0.40, 0.78	0.527	50	<0.001
	Peppermint EO	7	2.80	1.12, 4.48	0.001	65	0.009
	Thyme EO	6	0.84	-0.61, 2.30	0.256	0	0.678
	Ginger EO	3	-3.02	-11.91, 5.88	0.506	69	0.041
	Cinnamon EO	9	0.86	-0.40, 2.12	0.181	59	0.013
	Oregano EO	16	-2.03	-3.32, -0.75	0.002	74	<0.001
	Liquidambar EO	10	2.87	1.13, 4.62	0.001	64	0.005
	Star anise EO	3	5.09	0.93, 9.24	0.016	86	0.001
	Satureja EO	10	4.357	-0.50, 9.21	0.078	98	<0.001
Broiler strains	Arbor Acres	24	0.438	-0.30, 1.18	0.244	56	<0.001
	Ross	72	1.00	-0.01, 2.00	0.052	91	<0.001
	Lohmann	3	-3.90	-6.81, -1.00	0.008	0	0.710
	Cobb	6	2.64	0.18, 5.10	0.035	69	0.006
	Hybro	4	-1.35	-3.68, 0.99	0.258	77	0.004
Rearing phase	Starter phase	31	0.53	-0.55, 1.62	0.337	93	<0.001
	Finisher phase	18	1.90	-1.51, 5.30	0.275	78	<0.001
	Overall phase	60	0.55	-0.42, 1.51	0.266	89	<0.001

Abdominal fat and blood lipid profiles

Table 7 shows the impact of covariates on the abdominal fat weight of broilers. Supplementation of EO at 51-100 and 101-200 mg/kg reduced the amount of fat accumulated in the abdominal region of broilers. Abdominal fat was not influenced in broilers fed 1-50 mg EO/kg. However, birds fed peppermint and blended EO significantly reduced abdominal fat. Broilers fed liquidambar EO had similar abdominal fat to the controls. Ross and Arbor Acres on EO diets had a lower abdominal fat weight than those on the control diets. The impact of covariates on blood TG values in broilers, as shown

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in *Table 8* revealed that broilers fed 51-100 mg EO/kg exhibited lower TG than those offered the control diets. Broilers fed 1-50, 101-200, 201-300, and 401-500 mg/kg, however, had comparable TG with those fed control diets. Broilers on dietary liquidambar and satureja EO intervention had statistically reduced TG compared to those fed ginger EO. In converse, TG was not affected in broilers fed thyme and blended EO. Likewise, TG was not affected by strain or rearing phase.

Table 5. *Effect of covariates on FCR in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	13	-0.05	-0.07, -0.02	0.001	59	0.003
	51-100	24	-0.04	-0.08, -0.01	0.012	92	<0.001
	101-200	22	-0.01	-0.03, 0.02	0.662	90	<0.001
	201-300	18	-0.17	-0.25, -0.09	<0.001	97	<0.001
	301-400	4	-0.01	-0.03, 0.01	0.228	0	0.896
	401-500	12	-0.13	-0.19, -0.07	<0.001	92	<0.001
	501-600	4	-0.03	-0.05, -0.01	0.002	0	0.655
	> 600	12	-0.06	-0.09, -0.04	<0.001	77	<0.001
Type of EO	Blended EO	42	-0.05	-0.06, -0.03	<0.001	89	<0.001
	Peppermint EO	7	-0.11	-0.21, -0.01	0.038	94	<0.001
	Thyme EO	8	0.04	-0.06, 0.14	0.410	78	<0.001
	Ginger EO	3	0.12	-0.40, 0.64	0.639	96	<0.001
	Cinnamon EO	9	0.03	-0.02, 0.08	0.225	87	<0.001
	Oregano EO	16	-0.05	-0.08, -0.03	<0.001	70	<0.001
	Liquidambar EO	9	-0.02	-0.04, 0.01	0.246	40	0.101
	Star anise EO	3	0.04	0.01, 0.07	0.002	0	0.676
	Satureja EO	12	-0.26	-0.35, -0.16	<0.001	98	<0.001
Strains	Arbor Acres	24	-0.02	-0.04; -0.01	0.006	64	<0.001
	Ross	63	-0.07	-0.10, -0.04	<0.001	96	<0.001
	Lohmann	3	-0.16	-0.22, -0.09	<0.001	47	0.150
	Cobb	15	-0.04	-0.07, -0.03	0.030	90	<0.001
	Hybro	4	-0.02	-0.07, 0.03	0.475	84	<0.001
Rearing phase	Starter phase	36	-0.05	-0.08, -0.03	<0.001	92	<0.001
	Finisher phase	20	-0.12	-0.18, 0.05	<0.001	98	<0.001
	Overall phase	53	-0.04	-0.07, -0.01	0.002	94	<0.001

Table 9 shows the influence of studied covariates on blood TC in broilers. TC was affected by dosage, with broilers fed 51-100 mg EO/kg having a higher value than those offered control diets. In contrast, TC was not affected in broilers fed 1-50, 101-200, 201-300, 401-500, and > 600 mg EO/kg. In comparison with the control group, TC was reduced in broilers offered ginger and liquidambar EO. During the production phase, Ross strain fed EO diets had reduced TC when compared with the controls. The effect of covariates on blood HDL in broilers fed EO-based diets as presented in *Table 10* showed that HDL was not affected by dosage and EO types. Ross in group fed EO diets during the overall production phase recorded higher HDL than the group fed control diets. *Table 11* shows the effect of covariates on LDL in broilers. The results showed that in broilers, dosage had no significant effect on LDL. In contrast, birds fed liquidambar and

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Satureja EO had significantly lower LDL than the controls, while those fed ginger and thyme EO had similar LDL as the controls. There was a strain and rearing phase effect on LDL in this study with Ross stain fed EO-supplemented diets during the overall production phase, showing lower HDL than the controls.

Table 6. *Effect of covariates on BWG in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	13	2.00	1.00, 3.00	<0.001	79	<0.001
	51-100	20	2.89	1.41, 4.37	<0.001	93	<0.001
	101-200	40	1.26	0.17, 2.35	0.024	94	<0.001
	201-300	18	2.99	0.27, 5.72	0.031	98	<0.001
	301-400	4	0.24	0.53, 1.01	0.044	0	0.997
	401-500	12	5.35	1.27, 9.44	0.010	99	<0.001
	501-600	3	0.88	-0.03, 1.79	0.058	0	0.928
	> 600	13	1.15	0.56, 1.74	<0.001	37	0.086
Type of EO	Blended EO	56	1.80	1.08, 2.51	<0.001	90	<0.001
	Peppermint EO	7	4.04	1.06, 7.01	0.008	96	<0.001
	Thyme EO	8	3.48	0.59, 6.37	0.018	88	<0.001
	Ginger EO	3	-2.98	-13.53, 7.57	0.580	96	<0.001
	Cinnamon EO	9	-0.03	-1.64, 1.59	0.973	87	<0.001
	Oregano EO	16	0.09	-0.53, 0.71	0.768	57	0.002
	Liquidambar EO	9	2.14	0.55, 3.73	0.008	87	<0.001
	Star anise EO	3	1.11	0.45, 1.78	<0.001	0	0.470
	Satureja EO	12	7.46	2.85, 12.07	0.002	99	<0.001
Broiler strains	Arbor Acres	24	1.06	0.58, 1.53	<0.001	49	0.004
	Ross	77	2.68	1.55, 3.80	<0.001	98	<0.001
	Lohmann	3	2.91	1.36, 4.47	<0.001	0	0.450
	Cobb	15	1.15	0.24, 2.06	0.013	80	<0.001
	Hybro	4	-0.20	-1.29, 0.90	0.725	59	0.065
Rearing phase	Starter phase	36	1.61	0.48, 2.74	0.005	97	<0.001
	Finisher phase	20	4.24	1.00, 7.48	0.010	97	<0.001
	Overall phase	67	1.74	0.71, 2.77	<0.001	94	<0.001

Table 7. *Effect of covariates on abdominal fat content in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	4	-0.16	-0.36, 0.05	0.128	99	<0.001
	51-100	7	-0.28	-0.39, -0.17	<0.001	97	<0.001
	101-200	5	-0.20	-0.35, -0.05	0.008	98	<0.001
Type of EO	Blended EO	11	-0.20	-0.29, -0.11	<0.001	98	<0.001
	Peppermint EO	4	-0.43	-0.51, -0.35	<0.001	91	<0.001
	Liquidambar EO	3	-0.13	-0.30, 0.04	0.122	98	<0.001
Broiler strains	Arbor Acres	8	-0.26	-0.37, -0.15	<0.001	98	<0.001
	Ross	14	-0.18	-0.28, -0.07	<0.001	98	<0.001

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***Table 8.** Effect of covariates on blood triglycerides in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	4	-3.54	-13.80, 6.72	0.499	95	<0.001
	51-100	7	-8.78	-16.06, -1.49	0.018	98	<0.001
	101-200	5	8.46	-2.59, 19.50	0.133	99	<0.001
	201-300	5	7.06	-4.43, 18.56	0.229	99	<0.001
	401-500	3	-12.53	-25.45, 0.43	0.058	99	<0.001
Type of EO	Blended EO	6	0.10	-3.51, 3.70	0.959	91	<0.001
	Thyme EO	4	8.35	-15.51, 32.21	0.493	99	<0.001
	Ginger EO	3	13.81	0.58, 27.04	0.041	97	<0.001
	Liquidambar EO	3	-1.35	-2.57, -0.12	0.031	0	0.494
	Satureja EO	4	-12.19	-21.97, -2.41	0.015	99	<0.001
Broiler strains	Arbor Acres	4	2.53	-2.25, 7.31	0.300	33	0.216
	Ross	18	-2.23	-8.29, 3.824	0.470	99	<0.001
Rearing phase	Overall phase	22	-1.474	-6.15, 3.20	0.537	99	<0.001

***Table 9.** Effect of covariates on blood total cholesterol levels in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	1-50	4	-9.03	-18.64, 0.57	0.065	91	<0.001
	51-100	8	-20.10	-30.05, -10.14	<0.001	97	<0.001
	101-200	5	1.12	-11.85, 14.09	0.866	97	<0.001
	201-300	5	-0.35	-8.73, 8.03	0.935	93	<0.001
	401-500	3	2.06	-3.08, 7.20	0.432	94	<0.001
	> 600	3	6.86	-2.31, 16.03	0.142	98	<0.001
Type of EO	Blended EO	9	2.83	-6.31, 11.98	0.544	98	<0.001
	Thyme EO	4	-1.76	-14.22, 10.71	0.783	93	<0.001
	Ginger EO	3	-4.54	-8.17, -0.91	0.014	0	0.769
	Liquidambar EO	3	-16.57	-22.58, -10.56	<0.001	85	<0.001
	Satureja EO	4	3.94	-0.84, 8.72	0.106	89	<0.001
Broiler strains	Arbor Acres	4	2.03	-3.23, 7.29	0.449	45	0.144
	Ross	20	-6.87	-8.52, -5.21	<0.001	97	<0.001
	Cobb	4	7.28	-5.97, 20.54	0.282	98	<0.001
Rearing phase	Overall phase	27	-3.53	-5.08, -1.97	<0.001	96	<0.001

Meta-regression and publication bias

Significant heterogeneity (I2 = 88 - 99%, P < 0.001; *Tables 2* and *3*) was detected in all the response parameters of interest, which could not be removed by subgroup analyses (*Tables 4–6*). Egger's regression asymmetry test as presented in *Tables 2* and *3* was not significant, implying no publication bias. The association between the measured

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outcomes and studied covariates is presented in *Table 12*. Meta-regression found significant relationships between aspects of measured outcomes and EO type. EO types explained 18%, 41%, 17%, 27%, and 75% of the heterogeneity reported in FI, FCR, BWG, abdominal fat, and TC. Significant association was also detected between FCR, and dosage used ($P < 0.001$). Dosage accounted for about 22% of heterogeneity detected in FCR. Meta-regression analysis was not conducted for TG, HDL, and LDL since Borenstein et al. (2009) recommended that meta-regression analysis should not apply to measured outcomes with <10 studies due to poor statistical power.

Table 10. *Effect of covariates on blood HDL in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	51-100	6	8.97	-0.09, 18.02	0.052	99	<0.001
	101-200	3	0.61	-4.78, 6.00	0.824	95	<0.001
	201-300	5	3.40	-3.12, 9.93	0.306	98	<0.001
	401-500	3	8.93	-8.94, 26.80	0.327	90	<0.001
Type of EO	Thyme EO	4	1.33	-1.93, 4.58	0.425	89	<0.001
	Ginger EO	3	5.98	-6.44, 18.40	0.345	99	<0.001
	Liquidambar EO	3	2.26	-2.28, 6.80	0.329	91	<0.001
	Satureja EO	4	7.24	-3.66, 18.13	0.193	99	<0.001
Broiler strains	Ross	19	5.70	1.48, 9.92	0.008	99	<0.001
Rearing phase	Overall phase	17	5.47	1.01, 9.93	0.016	99	<0.001

Table 11. *Effect of covariates on blood LDL in broilers on dietary EO supplementation*

Covariates	Subgroup	n	MD	95% CI	*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Dosage (mg/kg)	51-100	6	-14.72	-30.21, 0.78	0.063	99	<0.001
	101-200	3	0.738	-7.62, 9.09	0.863	95	<0.001
	201-300	4	0.085	-4.97, 5.14	0.974	96	<0.001
Type of EO	Thyme EO	4	-0.61	-13.41, 12.20	0.926	98	<0.001
	Ginger EO	3	2.35	-1.58, 6.28	0.241	64	0.061
	Liquidambar EO	3	-5.87	-6.91, -4.84	<0.001	46	0.159
	Satureja EO	3	-8.38	-11.54, -5.23	<0.001	96	<0.001
Broiler strains	Ross	17	-7.38	-11.69, -3.07	<0.001	99	<0.001
Rearing phase	Overall phase	17	-7.38	-11.69, -3.07	<0.001	99	<0.001

Discussion

Growth performance

Although the exact mode of action of EO on improving chicken productivity is not clear. However, it is suggested that EO enhances broiler performance by improving gut health, digestive enzyme secretion, and nutrient digestibility (Gopi et al., 2014; Al-Hijazeen et al., 2016). In the present meta-analysis, dietary EO intervention improved FI, BWG, and FCR in broilers. This is consistent with Khattak et al. (2014) and Giannenas

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et al. (2018), who found improved FI in broilers fed a basal diet with EO at 1.0 g/kg. In a more recent study, Huang et al. (2024) detected improvement in growth variables of broilers on dietary oregano, peppermint, and thyme EO supplementation at 200 mg/kg feed. The observed improvement in FI and FCR can be explained by the ability of EOs to stimulate the release of endogenous digestive juice and enzymes, resulting in increased feed consumption, and nutrient digestion (Hashemipour et al., 2016; Masouri et al., 2017). The significantly high BWG and superior FCR in broilers fed EO-supplemented diets in this study can be partly ascribed to the enhanced quality of the diets and improved utilization by the broilers. Another possible explanation for the positive effect of EO supplements on broiler growth dynamics as observed in the present meta-analysis could be attributed to the antimicrobial properties of EOs, which stimulate the growth of beneficial microbes in the chicken gut (Tiihonen et al., 2010; Mangalagiri et al., 2021). This result supports the views of other researchers (Gopi et al., 2014; Al-Hijazeen et al., 2016) that dietary EO supplementation encourages the growth of healthy microbes in the gut, leading to improved BWG. In contrast, Huang et al. (2024) disclosed that the inclusion of high doses of EO (400, 600, and 800 mg/kg) in broiler diets reduced growth performance parameters. In a similar feeding study, Khattak et al. (2014) found that growth performance in broilers was not affected by blended EO supplementation at 100-500 g/tonne feed. This variation in response may be due to differences in the type of EO used and quantity included in the diet, as corroborated by our meta-regression results. Other factors that may affect the response in growth performance to dietary EO are environmental conditions, such as housing temperature, relative humidity, and ventilation rate, which according to Baracho et al. (2019) affect broiler performance.

Table 12. *Relationships between measured traits and covariates*

Outcomes	Covariates	Q_M	df	*p*-value	R^2 (%)
Feed intake	Dosage	8.96	7	0.256	2
	Type of EO	34.1	12	<0.001	18
	Broiler strains	6.84	4	0.144	3
	Rearing phase	1.99	2	0.369	0
Feed conversion ratio	Dosage	34.8	7	<0.001	22
	Type of EO	78.5	12	<0.001	41
	Broiler strains	4.78	4	0.311	1
	Rearing phase	5.99	2	0.05	3
Average daily gain	Dosage	11.2	7	0.129	4
	Type of EO	35.8	12	<0.001	17
	Broiler strains	4.12	4	0.390	0
	Rearing phase	5.53	2	0.063	3
Abdominal fat	Dosage	1.5	7	0.982	0
	Type of EO	12.5	5	0.029	27
	Broiler strains	0.853	1	0.356	0
Total cholesterol	Dosage	9.69	5	0.0846	15
	Type of EO	77.9	8	<0.001	75
	Broiler strains	4.38	2	0.112	8
	Rearing phase	1.04	1	0.308	0

Q_M, coefficient of moderators df degree of freedom; R^2, the amount of heterogeneity accounted for

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Abdominal fat content and blood lipid profiles

Accumulation of excess fat in the abdominal region of broilers is one of the challenges faced by the broiler industry since it has been reported to reduce feed efficiency, carcass, and meat quality (Ogbuewu et al., 2022b). The significant reduction in abdominal fat in broilers fed EO diets in this study implied a decline in fat deposition in the meat, which could lead to the acceptability of the meat by consumers. Others (Attia et al., 2019; Elbaz et al., 2022; Ghazanfari et al., 2024) found a reduction effect of EO supplements on abdominal fat in broilers. In contrast, Ciftci et al. (2009) suggested that dietary cinnamon EO intervention at 1000 mg/kg for 30 days increased abdominal fat weight in one-day-old Ross 308 broilers. The disparity in results may be due to differences in husbandry conditions, age of broilers, diet composition, chicken genetics, type of EO used, and quantity of EO added to the feed. The exact mechanism by which EO reduces fat deposition in the abdominal region of broilers is unknown. However, future studies should be channeled in this direction.

To our understanding, this is the first meta-analysis to assess the effect of EO supplements on the lipid characteristics of broilers. In broilers, there are correlations between blood lipids and diet composition (Ogbuewu and Mbajiorgu, 2024). Broilers in the control and EO groups had similar blood TG levels, implying that EO had no detrimental impact on the production of triglyceride. This finding agrees with Mehr et al. (2014a, b), who recorded similar blood TG in broilers fed EO-based diets. Although the effect of EO supplementation on 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases was not analyzed in the present study, it is documented that EO bioactive substances inhibit the activities of hepatic HMG-CoA reductases in rat liver (Lee et al., 2003a). The significantly low blood TC levels in broilers fed EO intervention may be due to the inhibitory actions of EO bioactive compounds on HMG-CoA reductases and other enzymes required for cholesterol biosynthesis in the liver. In the present meta-analysis, EO supplementation improved blood HDL and LDL levels in broilers. This implies that EO supplementation enhances the production of beneficial cholesterol (HDL) and reduces the production of undesirable cholesterol in broilers. In a similar experiment, Lee et al. (2003b) and Reis et al. (2018) found that the inclusion of EO in broiler diets did not affect blood cholesterol levels. However, the disparity in response may be attributed to differences in types and quantity of EO added to the ration (Hippenstiel et al., 2011; Hafeez et al., 2016). Other variables that may influence the response in blood lipid profiles to the inclusion of EO in the feed are diet constituents and sanitary conditions of the environment where the birds were kept.

Analysis of covariates and meta-regression

Subgroup analysis revealed that supplementation of EO at 1-50 and 51-100 mg/kg in broiler diets increased FI, suggesting that 1-100 mg EO/kg could be the level that optimized FI in broilers. In converse, broilers fed EO at the other supplemental levels in this meta-analysis had comparable FI with the control. Results also suggest that broilers that received peppermint, liquidambar, and star anise EO consumed more feed than the control. Likewise, broilers fed peppermint, liquidambar, and star anise EO consumed more feed than those fed oregano EO and blended EO. This observation is in harmony with Akbari et al. (2016) and Abdel-Wareth et al. (2019), who highlighted the appetite-stimulating effect of menthol, the main bioactive substance in peppermint EO. Results showed that Cobb consumed more diets than Lohmann, which supports the earlier views

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of Sebola et al. (2015), that chicken genetics. However, this result should be discussed with caution since few studies were used for their computation.

Results showed that the rearing phase and dosage had a significant effect on FCR and BWG in broilers. Broilers fed star anise EO-supplemented diets had significantly higher FCR than those fed peppermint, cinnamon, oregano, satureja, and blended EO. It is probable that the quantity of star anise EO added to the diet was insufficient to exert its positive influence on broiler FCR. However, this result needs to be interpreted with caution, as fewer studies were used for their computation. Broilers fed EO at 201-300 mg/kg had a higher magnitude of effect size (MD = 0.17) than other dose levels in the present study, implying that 201-300 mg EO/kg could be the level of EO that supported FCR. Results showed that broilers fed peppermint, thyme, liquidambar, star anise, satureja, and blended EO gained more weight than those fed the control diets. This finding agrees with Tiihonen et al. (2010), who discovered that EO blend enhanced BWG in broilers. A similar observation was made by Alcicek et al. (2003) in young broilers on blended EO supplementation. This improvement in BWG of broilers fed EO could be due to improved release of digestive enzymes, which in turn enhances feed efficiency, feed digestion, and nutrient uptake (Petrolli et al., 2012). In contrast, BWG was not affected in broilers fed ginger, cinnamon, and oregano EO, suggesting that ginger, cinnamon, and oregano EO supported BWG in broilers. Results reveal that EO supplementation improved FCR and BWG in Arbor Acres, Ross, Cobb, and Lohmann strains, but had no significant effect on the Hybro strain. This finding supports the view of Rondelli et al. (2003) and Baracho et al. (2019) that the genetics of a chicken affects its performance.

There is little or no study on the effect of broiler strains, rearing phase, type of EO, and administration dose of EO on abdominal fat in broilers fed EO-based diets in the literature. Evaluation of the effect of broiler strains, rearing phase, type of EO, and administration dose of EO on abdominal fat weight of broilers is important. The present study suggests that Ross and Arbor Acres broilers fed peppermint and blended EO at 51-100 and 101-200 mg/kg had significantly reduced abdominal fat weight than broilers fed control diets. This suggests that peppermint EO and its blends may reduce fat synthesis in tissues by stimulating bile acid production and secretion (Hu et al., 2015). In a similar study, Nehme et al. (2021) revealed that dietary EO supplementation reduced fat deposition in the abdominal region of chickens. Broilers fed 1-50 mg EO/kg exhibited comparable abdominal fat to the control, indicating that 1-50 mg EO/kg may be less than the amount needed to inhibit fat formation in the liver.

Broilers offered ginger and liquidambar EO at 51-100 mg/kg had significantly lower blood TG and TC content than birds fed control diets, suggesting the hypocholesterolemic and hypolipidemic effects of ginger and liquidambar EO in broilers. This is in harmony with Herve et al. (2019) who stated that the addition of ginger EO to the quail diet at 100 mg/kg for 63 days reduced TG and TC by 23% and 24%, respectively. Ginger and liquidambar EO may have achieved this by hindering the activity of acylCoA-cholesterol acyltransferase, which esterifies cholesterol in the tissue (Ciftci et al., 2010). The significantly low blood TG and TC recorded in broilers that received EO at 51-100 mg/kg indicates that 51-100 mg/kg may be the level that optimizes blood TG in broilers. This meta-analysis revealed that Ross broilers that received EO-based diets during the overall production phase had higher blood HDL than the control, implying that EO can enhance the formation of HDL in the Ross strain. Likewise, this showed that LDL was reduced in the blood of Ross broilers fed liquidambar and satureja EO during the overall production phase compared to the control. This confirms the earlier report that supplementation of

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EO to livestock feed influences lipid metabolism in the tissue and organs, resulting in less accumulation of bad cholesterol (LDL-cholesterol) in the body (Barreto et al., 2008; Altop et al., 2017).

Conclusion

This study suggests that dietary EO supplementation improved growth performance parameters in broilers, implying that EO can be used as a natural growth promoter in the broiler industry. On the same hand, pooled results suggest that dietary EO intervention decreased blood levels of TC and LDL while increasing the blood levels of HDL. However, pooled analysis showed that blood TG levels in broilers were not affected by EO supplementation. There was evidence of significant heterogeneity in this meta-analysis, which subgroup analysis could not resolve. Meta-regression results showed that in broilers, dosage is a significant predictor of FCR, while type of EO is a significant predictor of FI, FCR, ADG, TC, and abdominal fat in broilers. Results revealed that dosage and type of EO explained most of the heterogeneity. It is recommended that future research should focus on determining other sources of heterogeneity in broilers fed EO-supplemented diets. The findings of this study will assist policymakers, farmers, and relevant stakeholders in making an informed decision on the use of EO as a performance-enhancing agent in broiler nutrition.

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