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Essential oils as a strategy to improve gut histomorphometry and performance of broilers: systematic review and meta-analysis

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Abstract

The interest in the search for alternatives to antibiotics in poultry production has been increasing, especially with the focus on essential oils due to their remarkable bioactive properties. This study aimed to investigate the effects of dietary supplementation of essential oils on the performance and gut morphometry of broilers, by using an approach of systematic review and meta-analysis. In the conduction of the systematic review, three electronic databases (PubMed, Science Direct and Scielo) were consulted in January 2023. Out of an initial amount of 162 papers, only 27 met the requisites to be included in the database. Furthermore, after the use of established criteria for the meta-analysis, only 16 papers were qualified for the evaluation of the aimed parameters. In the meta-analysis, it was observed that the supplementation had significant impact ($P < 0.05$) of 2.88% in weight gain, in comparison to the basal diet. In addition, the supplementation of essential oils significantly improved ($P < 0.05$) gut morphometry parameters such as villus height in the ileum (15.66% higher), and 8.26% increase in the villus height to crypt depth ratio in jejunum compared to the basal diet. Dietary essential oils improve the growth performance and gut histomorphometry of broilers, even when combined with antibiotics as growth promoters.

Introduction

In the last few years, the interest for the development of feed additives has significantly increased, with the essential oils (EOs) emerging as a promising alternative for the substitution of antibiotics in animal production (Kishawy *et al*., 2019; Mahgoub *et al*., 2019). This interest is based on the biological properties of the EOs, such as antimicrobial, antioxidant, anti-inflammatory and immunomodulatory activities (Donsì and Ferrari, 2016; Han *et al*., 2017; Lee *et al*., 2020; Su *et al*., 2021).

EOs are volatile and aromatic compounds extracted from plants, many of which show a broad spectrum of antimicrobial activity, affecting Gram-positive as well as Gram-negative bacteria (Su *et al*., 2021). EOs antimicrobial efficacy is intrinsically related to two important characteristics: their lipophilic character and the ability to penetrate in the membranes of bacterial cells, due to this lipophilic property (Bona *et al*., 2012; Chouhan *et al*., 2017; Abd El-Hack *et al*., 2022). When bacteria are exposed to EOs, they experience an increase in the permeability of their membranes, resulting in the cell lysis due to the release of cellular content (Dorman and Deans, 2000; Bona *et al*., 2012; Su *et al*., 2021). Also, this increase in permeability allows other active compounds present in EOs to penetrate the cells and bind to specific proteins, triggering a supplementary inhibitory action (Chouhan *et al*., 2017).

Depending on the composition of EOs or their combinations, we can observe a broad diversity of biological effects that go beyond the antibacterial activity. These effects include the reduction of oxidative stress in critical situations, leading to a reduction in the energy demand that is required for the antioxidant functions (Windisch *et al*., 2008; Mohebodini *et al*., 2021). Moreover, it is important to highlight the ability of some EOs to stimulate the secretion of digestive enzymes and endocrine hormones, resulting in further promotion of motility that is enhanced in the gastrointestinal system. This, in turn, contributes for the optimization of the processes of digestion and absorption of nutrients (Wade *et al*., 2018; Su *et al*., 2021), important to improve poultry performance. EOs have other benefits described in literature, such as antiviral, anti-helminthic and coccidiostatic activities (Basmacioğlu Malayoğlu *et al*., 2010).

Due to the diversity of bioactive compounds that are present in the EOs, and to the influence that biological factors can exert on their composition and combinations, as well as the diverse results related to the type of plant, harvest location and conditions; production methods, including types of extraction, distillation and stability; and storage conditions, such as light, temperature and storage time (Huyghebaert *et al*., 2011), conflicting results are observed in the use of EOs in broilers. Many authors suggested positive effects (Barbarestani *et al*., 2020; Su *et al*., 2021) in poultry performance, while others could not identify such effects and, in some cases, they even showed negative effects (Akbarian *et al*., 2015; Irawan *et al*., 2021). Based on the foregoing, this study aimed to evaluate the effect of EOs supplementation in broiler diets, and their effects on the animal development and gut morphometry, based on a systematic review with meta-analysis.

Materials and methods

Bibliographic research

This systematic review was conducted following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses – PRISMA (Page *et al*., 2021). Until March 2022, an electronic search was conducted in the PubMed, ScienceDirect and SciELO databases using the following keywords in English: broilers, chickens, oil, thymol, performance, blood (blood was used as one of the keywords because the initial objective was to evaluate, in addition to performance and intestinal morphometry, the blood profile as well; however, after data tabulation, it was observed that there were not enough data involving this parameter to perform statistical analysis) and morphology.

These keywords were used in various combinations. For the ScienceDirect platform, one combination was used: (broilers OR chickens) and ('oil') and ('thymol') and ('performance') and ('blood') and ('intestinal morphology'). Initially, the same combination ((broilers OR chickens) and ('oil') and ('thymol') and ('performance') and ('blood') and ('intestinal morphology')) was used on the PubMed and SciELO platforms; however, the search result was zero. For this reason, three other search combinations were used to expand the database. The keyword combinations used on the PubMed and SciELO platforms were: (broilers OR chickens) and ('oil') and ('thymol') and ('performance'); (broilers OR chickens) and ('thymol') and ('intestinal morphology'); and (broilers OR chickens) and ('thymol') and ('blood').

Eighty-eight articles were found, and after filtering by article titles, 26 articles were pre-selected for data tabulation and extraction. After a review of the obtained data, it was discussed among the researchers the need for a new search in the databases, which was conducted in December 2022. In this second search, the keywords set ((broilers OR chickens) and ('essential oil') and ('performance') and ('blood') and ('intestinal morphology')) was used. The difference in the keyword set aimed to obtain new articles to expand the database. Only articles involving research with broilers being supplemented with EOs were selected.

The screening carried out in the systematic review was through the exclusion of titles that were not aligned with the researchers' objectives, those that did not qualify as experimental studies, and those that were carried out *in vitro*, when they did not provide results in the type of quantitative data, as well as the papers that were duplicated between the databases. Moreover, the papers that approached EOs and other compounds, and the ones in which the animals were subjected to sanitation challenges.

Criteria for selecting papers and elaboration of databases

There were selected studies that included a control diet without supplementation of EOs, and a diet with the addition of EOs. The selected papers exhibit significant variation in the composition of bioactives, as well as their concentrations. Therefore, to assess the use of EOs, only the effects with or without supplementation were considered, similar to the approach taken by Moreira *et al*. (2020) when evaluating different amino acid blends.

No restrictions were imposed regarding the poultry's sex, strain, geographical latitude, season of the year, year the study was done or language used in the publishing of papers. In situations discrepancies between the documents were identified, all criteria were submitted to a detailed review and debated among researchers.

For the meta-analysis, information related to the performance of the animals was compiled (by including weight gain – WG, feed intake – FI and feed conversion – FC) and to the gut morphometry (covering measures such as villus height and crypt depth). This information was extracted from the tables present in the results section of each paper and organized into spreadsheets in Microsoft Excel (Arifin, 2016). Four distinct databases were created, one to evaluate the performance of the animals and the other to analyse the gut morphometry (duodenum, jejunum and ileum).

Evaluation of papers quality

After the selection criteria were applied, there was the evaluation of the quality of the papers, by taking into consideration the following criteria for the allocation of scores (Palencia *et al*., 2018; Moreira *et al*., 2020): (A) randomization: papers that described a randomized study were assigned a score of 2 points, while those that did not mention randomization, or where randomization was not clearly described in the text, were assigned 0 points; (B) detailing of density and creation: papers that mentioned the dimensions of the cages or pens for the calculation of density were allocated with 2 points, while the ones that did not mention this information obtained 0 points; (C) reference to the type of experimental unit (cage, pen box or stall): papers that mentioned the type of experimental unit revived 2 points, while those that did not mention it received 0 points; (D) reference to initial and final temperatures: papers that mentioned initial and final temperatures received 2 points, while those that did not received 0 points; (E) reference to lighting programme used: papers that mentioned the amount of lighting provided received 2 points, while those that did not mention received 0 points; (F) rearing broilers and mixed or single-sex: papers that related single-sex studies received 2 points, while those that related mixed-sex received 1 point; (G) nutritional phases: papers that mentioned three nutritional phases were assigned with 2 points, those with two phases got 1 point, and the ones that did not mention the nutritional phases or only cited the strain manual got 0 points; (H) definition of strain: papers that included the definition of strain received 1 point, while those that did not were not assigned.

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Each paper was classified based on the total score obtained after the sum of the scores assigned to each evaluated variable. This classification was used as a qualitative weighting criterion for the studies selected for this research. The quality criteria are necessary to evaluate the state of the art of the research line related to the objective in the article, considering the possible confounding factors in the analysis and conclusion of this work.

Statistical analysis

For the processing of statistical analysis, the data were tabbed by using electronic spreadsheets from Arifin (2016). The standard error of the mean (SEM) has been presented in studies. SEM involves a general estimate without distinguishing the group. Thus, to estimate the standard deviation (S) the relationship $\mathrm{SEM} = S/\sqrt{n}$ was used, with n being the number of repetitions in each group (McGrath *et al.*, 2023). The 'effect size' was determined by the mean difference between control treatment and the treatment with the inclusion of EOs, with confidence intervals of 95%. Heterogeneity was evaluated through the index of inconsistency (I^2) and Cochran's Q test (Davoodi *et al.*, 2022).

The I^2 statistics is a crucial measure in the meta-analysis in order to evaluate the aggregate studies. Derived from Cochran's Q test, and taking into consideration the number of involved studies, its P value was compared to the significance level of 5%, in order to determine, or not, heterogeneity. Moreover, the following classification of the I^2 statistics was used: values close to 0% show lack of heterogeneity, close to 25%, low heterogeneity, about 50%, moderate heterogeneity, and 75%, high heterogeneity among the studies. When heterogeneity is indicated, the model of random effect is the indicated one if compared to the model of fixed effect.

Upon finding a significant difference between the oil application and the control, a regression adjustment was performed using a mixed model (Irawan *et al.*, 2021). The model structure included a random effect associated with the variable Study, allowing for variation in both the intercept and the slope concerning Dose. Additionally, two fixed-effect models were considered: one with a linear effect of Dose and the other with both a linear and quadratic effect of this variable. These models were compared using the likelihood ratio test. The model was adjusted using the lme function from the nlme package (Pinheiro and Bates, 2006) in R software. All the statistical analysis was held at software R (R Core Team, 2023). Meta-analysis was done at the metalibrary (Balduzzi *et al.*, 2019).

Results

Systematic review

After searches in the three databases (PubMed, ScienceDirect and SciELO), it was observed that 78.40% of the papers were found at ScienceDirect. After the search, papers were excluded based on the pre-established criteria as follows: 5.48% of the papers were excluded because they were duplicate among the databases, 52.74% because of the title, 13.30% were reviews, 1.37% were *in vitro* studies, 2.05% of the studies did not clearly present the values of the analysed parameters, 6.16% were experiments in which substances other than EOs were evaluated and, finally, 18.49% were excluded because they were studies in which the animals were challenged, somehow, leaving 9.88% of the studies for the elaboration of the systematic review and meta-analysis (Table 1). The PRISMA flow diagram describes the stages of the study selection process and reasons for exclusion (Fig. 1).

Table 1. Papers screening

Searches in databases	Papers (*N*)	Papers (%)
PubMed	31	19.14
Science Direct	127	78.40
SciELO	4	2.47
Total of selected papers	162	100
Exclusion of papers	*Papers (N)*	*Papers (%)*
Duplicate	8	5.48
By the title	77	52.74
Not experimental research	20	13.70
In vitro study	2	1.37
Does not show results in numbers	3	2.05
Studies with blend of EO and other compounds	9	6.16
Studies in which animals were challenged	27	18.49
Total of excluded papers	146	90.12
Selected papers	*Papers (N)*	*Papers (%)*
	16	9.88

In the evaluation of the quality of the papers, the following percentage were observed of studies that were allocated a score of 2, according to measured parameters: (A) randomization (56.25%); (B) density or dimensions of cage or box (50.00%); (C) experimental unit (87.50%); (D) initial and final temperature (81.25%); (E) light provided (93.75%); (F) sexed (75.00%); (G) three nutritional phases (37.50); (H) defined strain (93.75%) (Table 2).

The 16 selected papers met the criteria of eligibility for the parameters to be evaluated, such as: effect of supplementation of EOs on the development at 42 days (WG, FI and FC) and gut morphometry (villus height, crypt depth and ratio between villus height and crypt depth) of the duodenum, jejunum and ileum.

Among the performance parameters with the treatment of EOs, it is weight gain (WG), where 14 studies reached the result and 57.00% of measurements were significant; for feed conversion (FC), the result was more expressive, from which the 13 studies that measured this parameter, 71.00% obtained significant result with the treatment of EOs. For feed intake (FI), the result was unimpressive, once, from the 13 studies that measured this parameter, only 7.60% reached a significant result with the treatment of EOs.

Regarding the gut morphometry, villus height was the parameter that best responded to EOs and in eight studies that measured this parameter in the duodenum, 50.00% differed from control treatment; in jejunum, out of the nine studies that measured this parameter, 33.00% showed significant result; in the ileum, the results were unimpressive, with only 11% of the nine studies that measured this parameter with significant result, with diets treated with EOs.

Among the 16 selected papers, it was possible to observe that Ross 308 strain represented 43.75% of the genetics found in

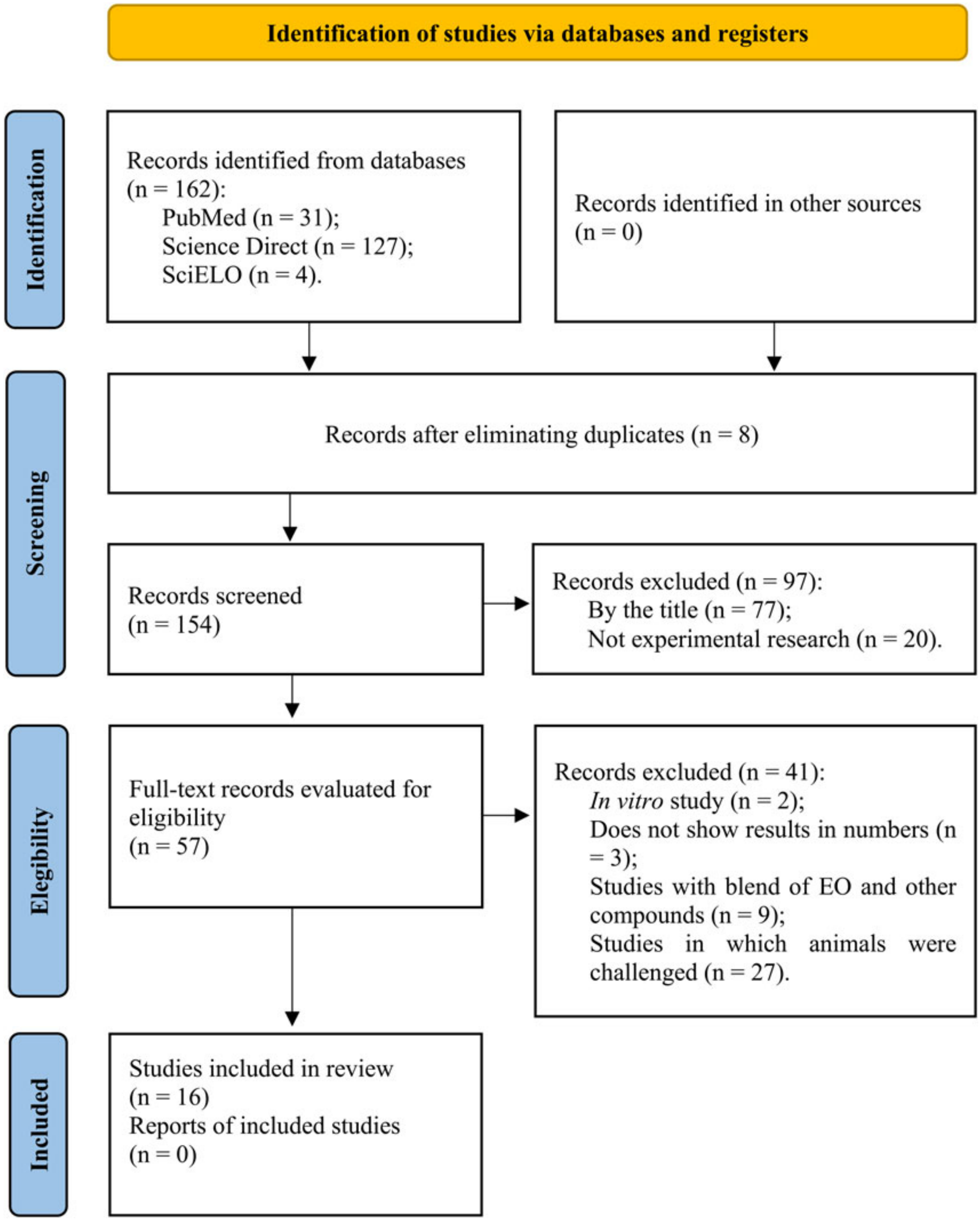

Figure 1. Modified PRISMA flow diagram (Page *et al*., 2021) with the systematic review search strategy and study selection.

studies, while the second largest participation of the strain was Arbor Acres with 31.25%. About the poultry sex, 68.75% were male flocks, 18.75% were mixed flocks and 12.50% of the studies did not describe the poultry sex. The mean inclusion of EOs, or its combinations was of 411 g/T feed. The protected EOs were present in 31.25% of papers. The treatment lasted from 1 to 42 days, for 62.50% of the analysed papers. Regarding the ages for the performance parameters measured, 81.25% were up to 42 days, and the same age for the observation of morphology in 56.25%, in a single collection or associated to one more data collection, varying from 21 to 28 days. Finally, the bioactive carvacrol, thymol and cinnamaldehyde were present in 56.25, 50.00 and 31.25%, respectively (Table 3).

Meta-analysis

In the duodenum, *P* values in Cochran's *Q* test for villus height, crypt depth and villus height:crypt depth ratio were 0.002, 0.030 and 0.760, respectively. The values for I^2 statistics for these parameters were 73, 61 and 0%, respectively. For villus height, the model of random effects is the most appropriate; additionally,

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Table 2. Evaluation of papers' quality according to pre-established criteria

Author/year	A	B	C	D	E	F	G	H	Total
Amerah *et al.* (2011)	2	0	2	2	2	2	1	2	13
Barbarestani *et al.* (2020)	0	2	2	2	2	2	1	2	13
Chowdhury *et al.* (2018)	2	2	2	2	2	1	2	2	15
Ding *et al.* (2022)	0	0	2	2	2	2	2	0	10
Du *et al.* (2016)	2	2	2	2	2	2	1	2	15
Emami *et al.* (2012)	0	2	2	2	2	2	1	2	13
García *et al.* (2007)	0	2	2	2	2	0	1	2	11
Hashemipour *et al.* (2013)	2	0	0	2	2	2	2	2	12
Hashemipour *et al.* (2014)	2	0	2	2	2	2	2	2	14
Hong *et al.* (2012)	0	2	2	0	2	1	1	2	10
Mohebodini *et al.* (2021)	2	2	2	2	2	2	2	2	16
Shirani *et al.* (2019)	2	0	2	2	2	2	2	2	14
Su *et al.* (2021)	0	2	2	2	2	2	1	2	13
Tsirtsikos *et al.* (2012)	0	0	0	0	0	2	0	2	4
Yang *et al.* (2019)	2	0	2	0	2	2	1	2	11
Zhang *et al.* (2021)	2	0	2	2	2	1	1	2	12

(A) Randomization: a randomized study scored 2 points, a non-randomized study (or when randomization was not clearly described in the text) scored 0 points; (B) studies that mentioned (density or) dimensions of cage or box for the calculation of density were allocated with 2 points, and when they did not, they scored 0 points; (C) studies that referred the type of experimental unit (cage, box or stall) were allocated with 2 points, and when they did not, they scored 0 points; (D) studies that mentioned initial and final temperature were allocated with 2 points, and when they did not, they scored 0 points; (E) studies that referred to the amount of provided light were allocated with 2 points, and when they did not, or only cited the lineage manual, they scored 0 points; (F) sexed studies were allocated with 2 points, and studies with mixed sexing got 1 point; (G) studies with three nutritional phases were allocated with 2 points, with two phases, they scored 1 point, and when they did not, or only cited the lineage manual, they scored 0 points; (H) studies with the definition of lineage scored 1 point, the study with no reference to the lineage did not score any points. For all the parameters that had not been mentioned, they were allocated 0 points.

the value of the ratio between the means was 39.44 ($P = 0.335$), and, then, there is no effect of supplementation of EOs. For crypt depth, the model of random effects is the most appropriate, and, also, the value of the mean difference ratio was −5.61 ($P = 0.451$), so, there is no effect of supplementation of EOs. For the villus height:crypt depth ratio, the model of fixed effects is the best one, the mean difference was −0.20 ($P = 0.094$), which suggests that there is no difference between supplementing or not with EO (Fig. 2).

The P values of Cochran's Q test for villus height, crypt depth and villus height:crypt depth ratio in jejunum were 0.560, 0.001 and 0.009, respectively. The I^2 statistical values for these parameters were 0, 84, 67%, respectively. For villus height, the model of fixed effects is the most appropriate one, the ratio mean difference was −28.15 ($P = 0.031$), suggesting that there is difference between supplementing or not with EOs. For crypt depth, the model of random effects is the best one, and, also, it suggests that the ratio mean difference was 10.81 ($P = 0.142$), and, then, there is no effect of supplementation with EOs. For the villus height:crypt depth ratio, the model of random effects is the most appropriate one, and the ratio mean difference was −0.45 ($P = 0.243$), which suggests that there is no difference between supplementing or not with EOs (Fig. 3).

In the ileum, the P values in the Cochran's Q test for villus height, crypt depth and villus height:crypt depth ratio were 0.054, 0.029 and 0.214, respectively. The values of the I^2 statistics for these parameters were 54, 60 and 31%, respectively. For villus height, the model of fixed effects is the most appropriate one, the ratio mean difference was −43.06 ($P = 0.004$), which suggests that there is difference between supplementing or not with EOs. For crypt depth, the model of random effects is the most appropriate one, the mean difference rate was 5.06 ($P = 0.282$), so, there is no effect of supplementation with EOs. For the villus height:crypt depth ratio, the model of random effects is the most appropriate one, the mean difference was −0.84 ($P = 0.001$), which suggests there is difference between supplementing or not with EOs (Fig. 4).

When the results of performance were evaluated, the P values of Cochran's Q test for final weight gain (WGP), daily weight gain (DWP), total feed intake (CF 0-42) and feed conversion (FC) were 0.767, 0.006, 0.025 and 0.001, respectively. The values of the I^2 statistics for these parameters were 0, 67, 60 and 99%, respectively. For variable WGP, the model of fixed effects is the best, the mean difference ratio was −77.40 ($P = 0.001$), which suggests there are differences between supplementing or not with EOs. For DWP, the model of random effects is the most appropriate one, the value of the mean difference was −1.35 ($P = 0.001$), so, there is an effect of supplementation with EOs. For CF 0-42, the model of random effects is the most appropriate one, the mean difference ratio was 22.42 ($P = 0.337$), which suggests there is no difference between supplementing or not with EOs. For FC, the model of random effects is the most appropriate one, the mean difference ratio was 0057 ($P = 0.129$), which suggests there is no difference between supplementing or not with EOs (Fig. 5).

Meta-regression

The likelihood ratio test indicated, in all adjustments, that the linear Dose model is the most appropriate. The results of the mixed-model estimates showed varying impacts of doses on the response

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Table 3. General abstract of periodicals

Author/year	Lineage	Sex	Inclusion EO (g/T)	P-EO	TD	MA/P	MA/M	Source of EO	Bioactive	Evaluated parameters
Amerah *et al.* (2011)	Ross 308	Males	100	Yes	1–35	35	NM	Enviva™ EO 101	Thymol and cinnamaldehyde	P
Barbarestani *et al.* (2020)	Arbor Acre	Males	300, 600	NI	0–42	42	42	*Lavandula angusti folia*	Linalool, acetate of cinnamaldehyde, eugenol, thymol, 1,8-cineole, α-pineno	P, D and J
Chowdhury *et al.* (2018)	Cobb 400	Mixed	300, 400, 600	NI	jan/39	NM	39	Allin Exporters	Linalool, acetate of cinnamaldehyde, eugenol, thymol, 1,8-cineole, α-pineno	D, J and I
Ding *et al.* (2022)	NI	Males	200, 400, 600	NI	jan/48	42	42	GuangZhou Wisdom Bio-Technology Co., Ltd	Thymol and carvacrol	P
Du *et al.* (2016)	Cobb 500	Males	60, 120, 240	Yes	jan/28	28	NI	Novus International Inc.	Thymol and carvacrol	I
Emami *et al.* (2012)	Ross 308	Males	200, 400	NI	jan/42	42	42	Peppermint	l-menthone and l-menthol	P, D and J
García *et al.* (2007)	Ross 308	Males	200	NI	1–42	42	NM	Oregano, cinnamon and pepper	Thymol, cinnamaldehyde and capsaicina	J
Hashemipour *et al.* (2013)	Ross 308	Males	60, 100, 200	Yes	0–42	42	NM	Next Enhance 150	Thymol and carvacrol	P
Hashemipour *et al.* (2014)	Ross 308	Males	100, 200	Yes	0–42	42	NM	Next Enhance 150	Thymol and carvacrol	P
Hong *et al.* (2012)	Arbor Acres	Mixed	125	NI	0–42	42	42	Biomin® PEP 125 aves	Carvacrol	D
Mohebodini *et al.* (2021)	Ross 308	Males	250, 500, 750, 1000	NI	1–42	42	NM	*Eucalyptus globulus*	1,8-cineole, α-pineno, α-terpineol, α-phellandrene cimeno, limonene	P
Shirani *et al.* (2019)	Ross 308	Males	111, 224, 337	NI	1–42	42	42	*Pulicaria gnaphalodes*	NI	P
Su *et al.* (2021)	Arbor Acres	Males	50, 100, 200, 400	NI	1–42	42	42	Tianjin NAER Bio-Tech Col., Ltd	Thymol, carvacrol and cinnamaldehyde	P, J and I
Tsirtsikos *et al.* (2012)	Cobb	Males	80,125, 180	NI	1–42	NM	42	Oregano, anise and limonene	Carvacrol and anetholes	D and I
Yang *et al.* (2019)	Arbor Acres	Males	50, 100, 200, 400, 800	NI	22–42	42	42	Cinnamon	Cinnamaldehyde	P, D, J and I
Zhang *et al.* (2021)	Arbor Acres	Mixed	200	Yes	1–42	42	42	Oregano	Thymol and carvacrol	P
	Ross: 43.75%	Males: 81,25%	Means: 262,77 g/T							

NI, non-identified; NM, not measured; EO, essential oil; P-EO, protected essential oil; TD, treatment duration; MA-P, measured age for performance; MA-M, morphology measured age; P, performance; D, duodenum; J, jejunum; I, ileum.

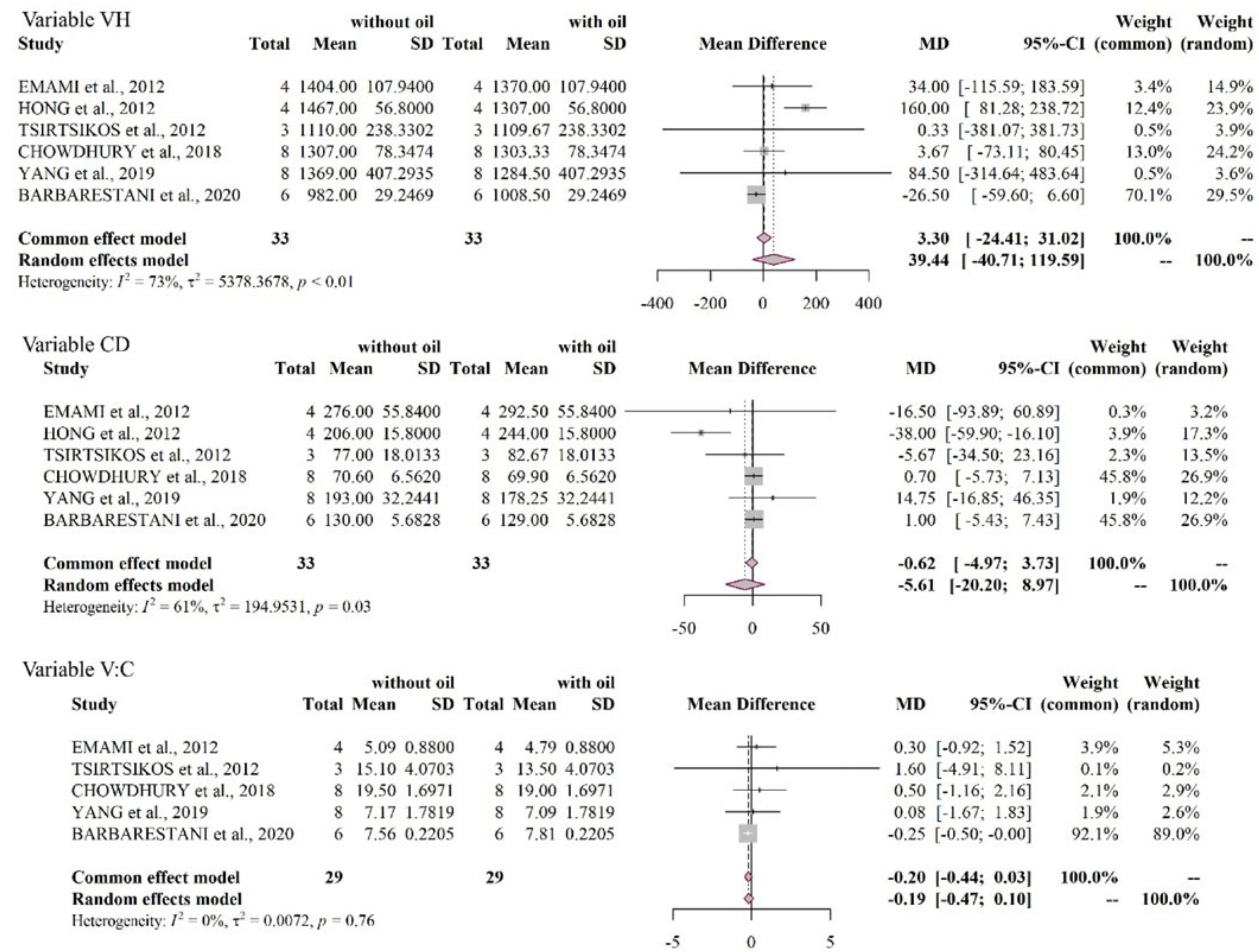

Figure 2. Forest plot of villus height (VC), crypt depth (CD) and the relationship between them (V:C) in the duodenum of broiler chickens as a function of dietary supplementation with essential oils.

variables. For the VH variables (ileum and jejunum) and CD (ileum), there was no significant dose effect ($P > 0.05$). However, for performance variables, WGP showed an intercept of 2379.194 (SE = 114.0602) and a significant slope ($P < 0.05$) of 0.144 (SE = 0.051), with an R^2 of 0.986. Similarly, performance measured by DWP had an intercept of 57.201 (SE = 3.233) and a significant slope ($P < 0.05$) of 0.004 (SE = 0.001), with an R^2 of 0.990 (Table 4).

Discussion

All the data on gut morphometry were collected from studies that measured this parameter after the poultry under treatment, at 21 days; this is because the broilers' gut reaches its maximum performance during the first 20 or 30 days of life; when there is a period of maturation that involves morphological adaptations that are relevant for the poultry (Maiorka, 2004).

The integrity of the cells that constitute the gut mucosa is one of the main factors for better absorption of nutrients, and, therefore, keep a healthy organism (Adedokun and Olojede, 2019). Thus, the gut immune system is its own epithelium, which is also responsible for the poultry development and growth. This gut barrier is formed by epithelial cells that are linked through joints, and provide impermeability to this layer of cells. In the area for the absorption of nutrients, the presence of villus and microvillus allows the maximization of the absorption, increasing the surface of epithelial layer (Celi *et al.*, 2017). The villus and crypts are two important components from the small intestine, and its geometry provides an indicator of the absorption ability (Heydarian *et al.*, 2020). The renewal of gut epithelium reflects the dynamic balance between the production of enterocytes in the crypts and its subsequent peeling of villus; therefore, villus height and crypt depth are available criteria to evaluate gut health and function (Su *et al.*, 2018). The villus height:crypt depth ratio (villus: crypt) is an indicator of the digestive ability of the small intestine. According to Luquetti (2005), a lower villus:crypt ratio means harmed villus and increased proliferative activity in the crypts, aiming to restore the epithelial form and function. On the other hand, the increase in this ratio corresponds to an increase in the nutrient's digestion and absorption (Montagne *et al.*, 2003), due to a bigger surface area.

In many studies, the effects of EOs were demonstrated in feed intake, nutrients metabolism, digestive secretions and growth (Krishan and Narang, 2014; Peng *et al.*, 2016; Mehdi *et al.*,

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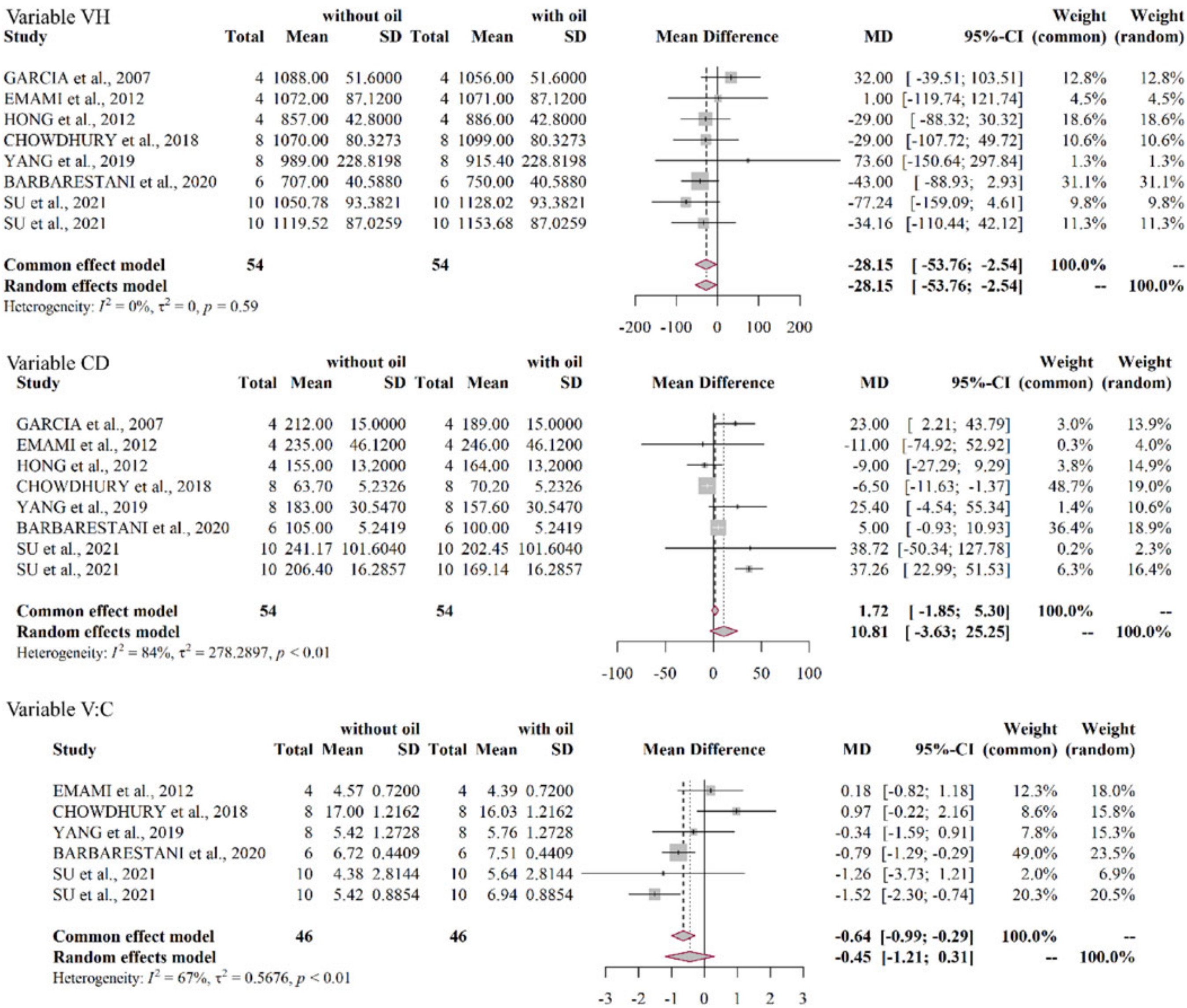

Figure 3. Forest plot of villus height (VC), crypt depth (CD) and the relationship between them (V:C) in the jejune of broiler chickens as a function of dietary supplementation with essential oils.

2018), as well as the effects on cultivable pathogens (Zhai *et al.*, 2018). However, other studies (Lee *et al.*, 2020; Mohebodini *et al.*, 2021) have shown that the response to feed supplementation of EOs in broilers improves the performance and feed efficiency, which has not been consistent, due to the dosage, source, type of EOs, diet and handling (Cross *et al.*, 2007).

The results show that diets supplemented with EOs had a significant effect, with better development of the ileum and jejunum. Therefore, the poultry that had been supplemented with EOs gained more weight, when compared to the poultry that had only been fed with a basal diet. Similar results for the morphometry of jejunum were also observed in the studies of Chowdhury *et al.* (2018); Barbarestani *et al.* (2020); Zhang *et al.* (2021); Su *et al.* (2020) and Ding *et al.* (2022). On the other hand, in the ileum, there were similar results observed to the study by Chowdhury *et al.* (2018). In the duodenum, there was no significant result with the supplementation of EOs, which can be attributed to the way the supplementation is carried out as in general, EOs are encapsulated to guarantee the efficacy of their compounds, which depend on stability, bioactivity and bio-availability of the active ingredients in the food matrix (Holkem *et al.*, 2015), and this is due to the volatility and ease of oxidation, which tend to suffer before the presence of light, air, humidity and high temperatures (Aburto *et al.*, 1998).

This microcapsule consists of a layer of the encapsulated agent, being, in general, constituted by polymeric material, which acts as a protective film, isolating the active substance, hindering its inadequate exposure. This membrane is torn under specific stimulus, releasing the substance in the place or in the ideal moment (do Carmo *et al.*, 2015), and in this case, the acting of EOs as a bioactive seems to freely happen in the jejunum and ileum, because the release of these compounds happens from the duodenum and, likewise, it is possible to infer that the results happened in the segments where the bioactive has longer time of action.

Beyond the microencapsulation, many factors may have influenced the results of meta-analysis, as a type of phytotherapy

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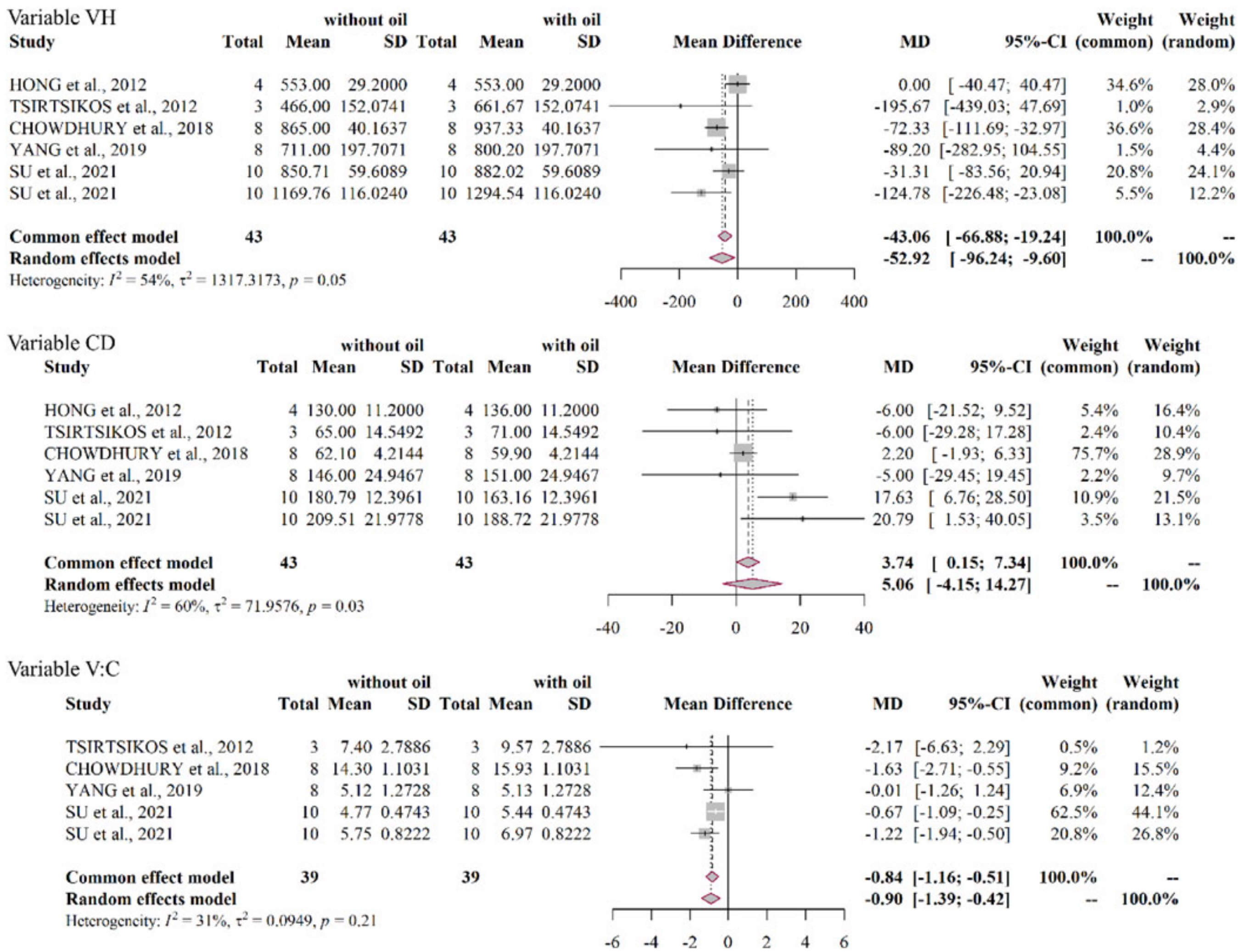

Figure 4. Forest plot of villus height (VC), crypt depth (CD) and the relationship between them (V:C) in the ileum of broiler chickens as a function of dietary supplementation with essential oils.

supplementation (dry herbs or plant extracts, e.g.). The concentration of active herbs in the plants can vary according to the used vegetative part, season, vegetative cycle, type of the soil where it was grown and the technique used for extraction (Windisch *et al.*, 2008). Therefore, according to Jamroz *et al.* (2005) the standardization of EOs is difficult (as it can be observed in table 5 that there are diverse sources of EOs, with different bioactives), as well as the standardization of their antimicrobial, antioxidant, immunomodulation activities and anti-inflammatory action.

Some studies show that the antimicrobial activity of EOs can induce a more balanced microbiota, because of the increase of the concentration of *Lactobacillus ssp*. and the decrease of *coliforms* and *E. coli* in broilers (Cetin *et al.*, 2016; Liu *et al.*, 2017; Giannenas *et al.*, 2018). The stimulatory effects of phytogenic additives in the gut secretion of mucus can prevent the adherence of pathogens to the mucosa gut (Jamroz *et al.*, 2006). On the other hand, beneficial bacteria such as the lactic ones can stimulate the increase of calceiform cells that are involved in the secretion of mucin. The layer of gut mucus plays a fundamental role in the hindering of the adherence of pathogens to gut epithelial cells, which, consequently, reduces the incidence of their toxic effects (Baurhoo *et al.*, 2009; Kim and Ho, 2010) and provides means for the increase of gut villus. In addition, the antimicrobial activity can be corroborated by the findings of Trombetta *et al.* (2005) and Devi *et al.* (2010), who affirmed that the group carbonyl of the *Cinnamaldehyde* is linked to the proteins, hindering the action of the enzyme and creating pores in the cellular membrane, and thymol and eugenol induce their antimicrobial action through a disorder of lipid fraction in the plasmatic membrane of the microorganisms, resulting in changes in the permeability of the membrane, and the extravasation of intracellular materials, till the possible cellular death.

Another property of EOs that may explain the results in this study is this antioxidant action, because, during the digestive processes, oxygen radicals are released, and they hinder the gut mucosa. The EOs protect the villi from oxidative damage. Nevertheless, bioactive substances that stimulate the activity of oxidative enzymes avoid damages to villus (Chowdhury *et al.*, 2018). The proximity of the mucosa surface and the gut content can motivate the oxidative stress which is caused by the digestive process (Windisch *et al.*, 2008). In this sense, phytogenic additives can positively affect the activity of antioxidant enzymes which, in turn, reduces the production of reactive species of oxygen, known

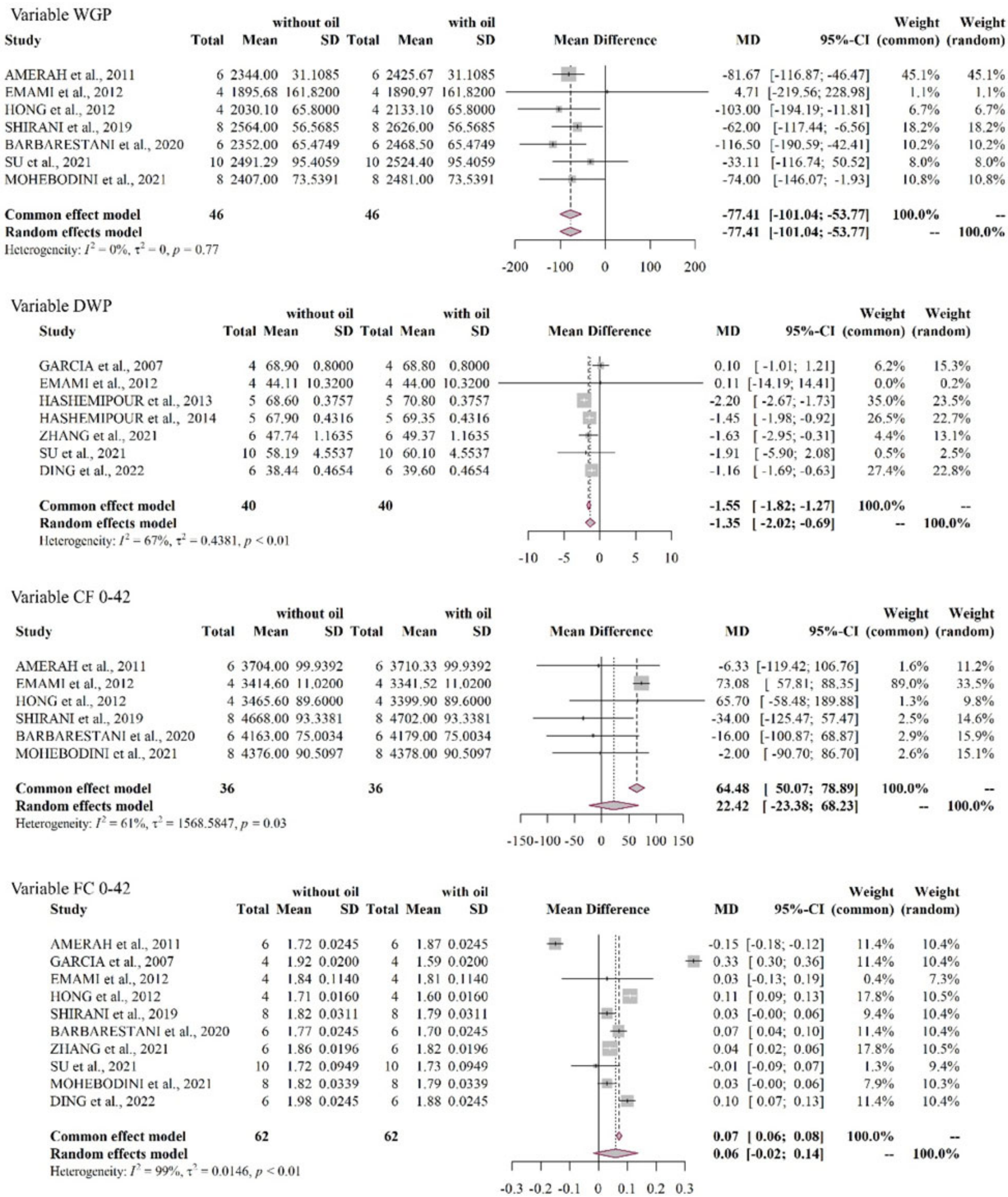

Figure 5. Forest plot of weight gain per phase (WGP), daily weight gain (DWP), feed consumption per phase (CF) and feed conversion (FC) as a function of the use of essential oils in the diet of broiler chickens.

as inflammatory factors in tissues and cells, by causing gut atrophy and disorder of the gut epithelial barrier (Moretti *et al.*, 2018). Excessive oxidative stress can cause gut inflammation and, even, cellular apoptosis in the tissue, following the dysfunctions (Xue *et al.*, 2020). Therefore, the antimicrobial and antioxidant properties of EOs can stimulate a healthy microbiota and consequently, the improvement in the immunological system of the gut mucosa, which has the duty of eliminating potential pathogens, keeping a relation which is mutually beneficial with the commensal microbiota (Liu *et al.*, 2020); the consequence can be a better availability of nutrients, resulting in efficiency in the feed conversion and weight gain.

In summary, this work represents significant advances in the use of EOs in broiler chickens. However, it is important to note

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Table 4. Regression equations on the effect of essential oils dose (g/T of diet) on production villus height (VH), crypt depth (CD), weight gain per phase (WGP) and daily weight gain (DWP) of broiler chickens

Response variables	Intercept (SE)	Slope (SE)	pLR	R^2	AIC	BIC
VH (ileo)	783.719* (144.616)	0.125 (0.122)	0.080	0.930	296.930	303.743
VH (jejuno)	993.574*(59.859)	0.052 (0.062)	0.357	0.818	341.393	349.387
CD (ileo)	7.421* (1.993)	0.001 (0.001)	0.186	0.963	94.684	101.497
WGP (performance)	2379.194* (114.060)	0.144* (0.051)	0.575	0.986	463.680	473.506
DWP (performance)	57.201* (3.233)	0.004* (0.001)	0.636	0.990	183.855	193.680

*Significant by *t*-test at 5%; pLR *P* value of the likelihood ratio test for comparison between the linear and quadratic model; R^2 represents the variance explained by the entire model, including both fixed and random effects (Nakagawa *et al*., 2017); AIC, Akaike information criterion; BIC, Bayesian information criterion; thy, thymol; car, carvacrol; cin, cinnamaldehyde; men, menthol; cap, capsaicina; cine, 1,8-cineole; pin, α-pineno; ter, α-terpineol; phe, α-phellandrene; cim, cimeno; lim, limonene.

that applying the article selection criteria revealed some limitations in the database for meta-analysis and meta-regression. In the meta-analysis, considering the presence or absence of EOs in the broiler diet, it was not possible to include the different doses reported in the papers within the statistical model. In the meta-regression, when considering the doses used in the studies, there is no standardization of the composition of the bioactives or their proportions within the compound. At present, the state of the art in this field requires a larger number of publications to address these limiting factors, although this study provides potential indications of which bioactives and concentrations may be studied or utilized.

Conclusion

The use of EOs in broiler diets has been proven, through this study of systematic review with meta-analysis, to be a supplementary tool to act in the improvement of feed efficiency and in the integrity of gut mucosa.

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Author contributions. Roberto Rocha, Pedro Fidelis and Andreia Massuquetto collected the data for this study; Sérgio Turra, Cláudia Lopes, Elias Medeiros and Rennan Moreira conducted the statistical analyses; Roberto Rocha, Camilla Silva and Marcelle Araújo wrote the initial draft of this manuscript; Rennan Moreira and Roberto Rocha collaborated in interpreting the results; Camilla Silva and Rennan Moreira finalized the manuscript. Both authors have read and approved the finalized manuscript.

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