Essential oils as growth-promoting additives on performance, nutrient digestibility, cecal microbes, and serum metabolites of broiler chickens: a meta-analysis

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Objective: The purpose of this meta-analysis was to evaluate the effect of dietary essential oils (EOs) on productive performance, nutrient digestibility, and serum metabolite profiles of broiler chickens and to compare their effectiveness as growth-promoting additives against antibiotics.

Methods: Peer-reviewed articles were retrieved from Web of Science, Science Direct, PubMed, and Google scholar and selected based on pre-determined criteria. A total of 41 articles containing 55 experiments with 163 treatment units were eligible for analyses. Data were subjected to a meta-analysis based on mixed model methodology considering the doses of EOs as fixed effects and the different studies as random effects.

Results: Results showed a linear increase (p<0.001) on body weight gain (BWG) where Antibiotics (FCR) and average daily feed intake decreased (p<0.001) linearly with an increasing dose of EOs. Positive effects were observed on the increased (p<0.01) digestibility of dry matter, crude protein, ether extract, and cecal Lactobacillus while Escherichia coli (E. coli) population in the cecum decreased (p<0.001) linearly. There was a quadratic effect on the weight of gizzard (p<0.01), spleen (p<0.05), bursa of fabricius (p<0.001), and liver (p<0.10) while carcass, abdominal fat, and pancreas increased (p<0.01) linearly. The dose of EOs linearly increased high density lipoprotein, glucose, protein, and globulin concentrations (p<0.01). In comparison to control and antibiotics, all type of EOs significantly reduced (p<0.001) FCR and tended to increase (p<0.1) BWG and final body weight. Cinnamaldehyde-compound was the only EOs type showing a tendency to increase (p<0.1) carcass weight, albumin, and protein of serum metabolites while this EOs together with EOs-Blend 1 decreased (p<0.01) E. coli population. Low density lipoprotein concentration decreased (p<0.05) with antibiotics and carvacrol-based compound when compared to the control group.

Conclusion: This evidence confirms that EOs are suitable to be used as growth promoters and their economical benefit appears to be promising.

Keywords: Antibiotics; Broiler; Essential Oils; Growth Promoter; Meta-analysis; Production Performance

INTRODUCTION

Global pressure to replace the use of antibiotics as growth promoters (AGPs) with safe feed additives in the broiler industry has led researchers to conduct a massive exploration in utilizing natural substance-based additives. It is primarily due to the AGPs restricted use by the European Union since 2006 concerning antimicrobial resistance, human health, and sustainability [1]. Essential oils (EOs), volatile properties derived from plant materials by mainly steam distillation method, are among promising growth enhancers in broiler chickens that received a growing interest among scholars and industrial stakeholders.
EOs are formed by dozens of complex mixture components [2] that can be classified into a group of terpenoids (menthol, linalool, geraniol, borneol, α-terpineol) and a group of low molecular weight aliphatic hydrocarbons (thymol, carvacrol, eugenol, cinnamaldehyde). The advantageous effects of EOs are associated with their role on many metabolic pathways, including on lipid metabolism, stimulate digestive enzyme secretion and activity, act as antimicrobial, and enhance gut integrity of chickens [3,4] leading to improve broiler performance in general.

However, inconsistent results among past and present studies are identified to be conflicting. For instance, there were positive effects of EOs on broiler performance as indicated with improving body weight gain (BWG) [5,6], feed conversion ratio (FCR) [7], enzyme secretion [8], and nutrients digestibility [9-11]. In addition, previous report also revealed that EOs supplementation effectively replaced the use of AGP [12]. On the other hand, there were contrary reports where several authors suggested no effect of dietary EOs on broiler productive performance [13,14] or even had lower weight gain compared to those not receiving AGP or EOs [15]. Major explanations from these contradictory findings have been attributed to the different of EOs active components used in the individual study, their natural origin such as plant source, plant part, geographical condition, and also environmental and physiological factors of the animal used [3,11]. Indeed, the mode of action from specific EOs molecules is limited to elucidate different results from a number of studies. The magnitude of the biological effect of EOs varies depending on the complex chemical structure of EOs.

Comprehensive reviews describing the main effect and mode of action of EOs on poultry have been provided [3,4,17]. However, to our knowledge, the effects of EOs on broiler chickens have not been summarized quantitatively to date. Data from available empirical studies can be integrated and quantified using a robust statistical method, i.e., the meta-analysis because the method considers heterogeneity among individual studies which increases the power to calculate the treatment effect [18,19]. Therefore, this study aimed to investigate the efficacy of the application of EOs as alternative growth-promoting additives in the diet on the productive performance of broiler chickens.

MATERIALS AND METHODS

Database development and inclusion criteria
A database was developed based on scientific publications available online at several search engines such as Web of Science, Science Direct, and PubMed. Keywords used in this study were “broiler performance” and “essential oil” or “phytogenic”. To specify the result of the browsing process, we used several filters available on the website such as type of article, range of year of publication, and journal name. Journal name filter was used to exclude irrelevant journals that appeared during searching the database such as journal related to aquaculture and food science. All relevant titles of publication from the respective websites were further imported into the reference manager for selection purposes.

In total, 124 published articles were identified to match the purpose of this study based on the title of the article. These data were further assessed with qualification criteria developed according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to ensure the quality of the meta-analysis [20]. Assessment criteria were, i) research articles published between 2006 to 2019 from a peer-reviewed journal, ii) in vivo trials containing control group and EOs treatment group, iii) articles not containing antibiotics used as a growth promoter in the control or EOs group except for the AGP used as a positive control, iv) articles reporting experimental periods, and at least performance parameters of final body weight (BW) or weight gain and feed intake or FCR, and v) articles reporting the EOs sources or major type of EOs and supplementation dose.

In addition, studies conducted before 2006 were excluded because it was the period in which the use of AGP in feed was permitted thereby difficult to measure the only effect of EOs inclusion. The EOs may also have been supplemented through feed or drinking water. In this study, only those administered in feed were integrated into the database. This meta-analysis also ruled out the bacterial challenged studies because they were not the focus of the current study. Because other substances such as organic acids can potentially interfere with the effect of EOs, studies that used organic acid products containing EOs compounds were excluded. Additionally, EOs treatments with ≥1,000 mg/kg were disregarded because the number of these categories was small (n total of this category = 9 observations). Finally, 41 studies comprising 55 experiments and 163 treatment units were eligible and therefore used for the analysis. Details for the selection process of database development are provided in Figure 1 while the summary of the studies is presented in Table 1.

Data extraction
The information about the journal, authors, year of study, broiler used (including strain and sex), diet type, length of study, treatment, type and composition of EOs, doses of EOs, nutritional specification of the diet, and the mean value of each parameter contained in the study was recorded in a spreadsheet. The parameters included in the database were productive performance (BW, BWG, feed intake (FI), FCR, average daily feed intake [ADFI]), nutrients digestibility (dry matter [DM], organic matter [OM], crude protein [CP], crude
Description of the database

This study included 16,221 broiler chickens averaging 395 (±87.7) birds per study. The strain used was dominated by Ross 308 which accounted for 73% while Cobb 500 was 17% and the other 10% were Arbor Across and Hubbard. All studies described broiler chickens’ sex used (of which 68% were male, 17% mixed, 10% unsex, and the rest was female). As much as 70.7% of all studies used maize – soybean meal based diet. The average duration of the evaluated study was 36 d, the maximum length was 46 d, and the minimum was 9 d. In 90.2% of the experiments, birds received starter diet, while grower and finisher diets were offered to 54.6% and 58.9% of the experiments, respectively. Nutrient specifications of all experiments were summarized for metabolizable energy (ME, kcal/kg), CP (%), and total lysine (%). As given in Table 2, the average of ME, CP, EE, and lysine of the diet provided to the starter, grower, and finisher phases were appropriate with the nutrient requirement of broiler chickens according to NRC [21].

The EOs are a complex mixture of a variety of bioactive compounds. In application, it is largely administered with one to three major components and some trace bioactive compounds, thus the term of EOs is used onward regardless...
of their specific composition. The EOs inclusion ranged from 0 (control) to 750 mg/kg and averaged at 112 (±149) mg/kg. The EOs included in the database were given in specific major component of EOs (thymol, carvacrol, cinnamaldehyde), a blend of EOs including as commercial EOs (thymol, carvacrol, eugenol, piperrine, cinnamaldehyde, anethole, menthol, y-terpinene, limonene, α-turmerone, p-cymene, camphor, α-pipene, d-limonene, linalool), or given as EOs extracted from specific plant sources (oregano EOs, thyme EOs, mint EOs, rosemary EOs, star anise EOs, basil EOs, caraway EOs, laurel EOs, lemon EOs, sage EOs, tea EOs, turmeric EOs, clove EOs). Antibiotics used as a positive control in the studies were salinomycin, zinc bacitracin, avilamycin, chlortetracycline, virginiamycin, and commercial anticoccidial (Cygro).

**Table 1.** Description of the studies included in the database

| Authors | Birds (n) | Period (d) | Essential oils | Dose (mg/kg) |
|---------|-----------|------------|----------------|--------------|
| Loh et al [56] | 96 | 0-21 | Blend (thy, ane) | 0-150 |
| Ciftci et al [57] | 240 | 5-42 | Cinnamon EOs | 0-500 |
| Isabel and Santos [58] | 660 | 0-46 | Blend (clove & cinnamon EOs) | 0-100 |
| Malayoglu et al [30] | 450 | 0-21 | oregano EOs | 0-500 |
| Tihtonen et al [51] | 720 | 0-42 | Blend (thy, cin) | 0-20 |
| Amad et al [9] | 528 | 0-42 | Blend (thy, ane) | 0-750 |
| Amerah et al [59] | 192 | 0-35 | Blend (cin, thy) | 0-100 |
| Alp et al [60] | 1200 | 0-42 | Oregano EOs | 0-300 |
| Bravo et al [61] | 240 | 0-21 | Blend (car, cin) | 0-100 |
| Bozkurt et al [62] | 1248 | 0-42 | Blend (car, 1,8-cin, cam, thy) | 0-48 |
| Engberg et al [63] | 640 | 0-35 | Artemisia annua oil EOs | 0-500 |
| Mueller et al [64] | 240 | 0-35 | Blend (turmeric, oregano, thyme, rosemary EOs) | 0-150 |
| Hashemipour et al [27] | 240 | 0-42 | Blend (thy, car) | 0-200 |
| Sarica and Corduk [65] | 135 | 0-21 | Oregano EOs, pepper EOs | 0-250 |
| Betancourt et al [66] | 750 | 0-42 | Oregano EOs | 0-200 |
| Habibi et al [67] | 168 | 0-49 | Ginger root EOs (zin, β-ses, sab, ar-cur, β-bis) | 0-150 |
| Hurmer et al [68] | 432 | 0-35 | Blend (thy, ane) | 0-150 |
| Khattak et al [5] | 960 | 0-42 | Blend (EOs from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme) | 0-60 |
| Ghazi et al [69] | 120 | 0-42 | Oregano EOs | 0-250 |
| Pirgozliev et al [70] | 310 | 0-21 | Blend (thy, eug, pip) | 0-300 |
| Aristimunha et al [12] | 930 | 0-42 | Blend (men, ane) | 0-100 |
| Hafeez et al [71] | 600 | 0-42 | Blend (car, cum, eug) | 0-300 |
| Baarmacioglu-Malayoglu et al [10] | 240 | 0-42 | Blend (thy, ane) | 0-150 |
| Kim et al [6] | 840 | 0-35 | Star anise EOs (ane) | 0-200 |
| Ding et al [72] | 192 | 0-42 | Oregano EOs, pepper EOs | 0-150 |
| Masouri et al [33] | 288 | 0-42 | Khuzistanica EOs (car) | 0-500 |
| Mohiti-Asli and Ghanaatparast-Rashti [73] | 200 | 0-42 | Blend (oregano, anise and citrus peel) | 0-150 |
| Paraskeuas et al [74] | 150 | 0-42 | Blend (men, ane, eug) | 0-150 |
| Ri et al [75] | 180 | 0-42 | Sweet gum EOs | 0-162.2 |
| Altop et al [28] | 375 | 0-42 | Blend (β-car, men) | 0-500 |
| Giannenas et al [76] | 320 | 0-42 | Blend (β-car, men) | 0-150 |
| Hosseini and Meimandipour [77] | 150 | 0-42 | Thyme EOs | 0-80 |
| Mohiti-Asli and Ghanaatparast-Rashti [78] | 200 | 0-42 | Blend (oregano, anise and citrus peel) and oregano | 0-500 |
| Pirgozliev et al [7] | 256 | 0-21 | Blend (cin, car, cap) | 0-100 |
| Park and Kim [38] | 360 | 0-42 | Blend (car, eug, pip) | 0-300 |
| Reis et al [52] | 240 | 0-42 | Blend (car, thy, cin) | 0-500 |
| Abdel-Wareth et al [25] | 384 | 0-35 | peppermint EOs and men | 0-222 |
| Attia et al [11] | 216 | 0-36 | Blend (cin, thy) | 0-150 |
| Galli et al [53] | 135 | 0-44 | Blend (car, thy, cin) | 0-100 |
| Nouri [79] | 300 | 0-14 | mint EOs, thyme EOs, cinnamon EOs | 0-55 |
| Placha et al [80] | 96 | 0-28 | Thyme EOs | 0-100 |

EOs, essential oils; thy, thymol; ane, anethole; car, carvacrol; cin, cinnamaldehyde; 1,8-cin, 1,8-cineole; zin, zingiberene; β-ses, β-sesquiphellandrene; sab, sabinene; ar-cur, ar-curcumene; β-bis, β-bisabolene, eug, eugenol, pip, piperrine; men, menthol; cum, cinnamaldehyde; β-car, β-caryophyllene; cap, capsicum oleoresin.
model analysis according to St-Pierre [18]. The SAS software was employed to analyze the data following the PROC MIXED procedure (SAS Studio 3.8, University Edition, 2018). The EOs were considered as fixed effects whereas the different studies were taken into account as random effects. The model applied was:

\[ Y_{ij} = B_0 + B_1 X_{ij} + B_2 X_{ij}^2 + S_{ij} + b_i + e_{ij} \]

where \( Y_{ij} \) = the expected output for dependent variable \( Y \) at level \( j \) from the variable \( X \) as a continuous variable in the study \( i \), \( B_0 = \) overall intercept across experiments (fixed effect), \( B_1 = \) linear regression coefficient of \( Y \) on \( X \) (fixed effect), \( B_2 = \) quadratic regression coefficient of \( Y \) on \( X \) (fixed effect), \( X_{ij} \) = value of the continuous predictor variable \( X \) (doses of EOs administration), \( S_{ij} = \) random effect of experiment \( i \), \( b_i = \) random effect of experiment \( i \) on the regression coefficient of \( Y \) on \( X \) in experiment \( i \) and \( e_{ij} = \) the residual error. In the statement CLASS, the “study” variable was declared. Data were weighted by the number of replicates in each study. Additionally, an unstructured variance – covariance matrix (type = un) was performed at the random effect part of the model to avoid a positive correlation between intercepts and slopes [18]. Regarding the continuous predictor, p-values for intercepts and slopes, Akaike’s information criterion, and root mean square error were used for model statistics [22,23]. Meanwhile, to test the effectiveness of type of EOs, we categorized the EOs according to their major compound and antimicrobial activity following the classification and justification from the evidence of previous reports [3,24]. Here, the EOs groups possible to compare were thymol-based compound (thy-BC), carvacrol-based compound (car-BC), cinnamaldehyde-based compound (cin-BC), and menthol-based compound (men-BC). Treatments containing one or more combinations of these compounds except for menthol were grouped as EOs-Blend 1 (EOB 1) and the other containing multiple compound of terpenoids (linalool, geraniol, thujaanol, borneol, menthol, citronellol, terpineol) was grouped as EOs-Blend 2 (EOB 2). We did not group a single compound from terpenoids except for menthol because the sample size was too small. As a result, there were eight treatment groups consisting of six EOs categories as moderating variables, AGP group, and control group. The categorical analysis was performed according to the following model:

\[ Y_{ij} = \mu + S_i + r_j + \tau_{ij} + e_{ij} \]

where \( Y_{ij} = \) the expected output for dependent variable \( Y \), \( \mu = \) overall mean, \( S_i = \) random effect of \( i \) study, \( r_j = \) fixed effect of the \( j \) level, \( \tau_{ij} = \) random interaction between \( i \) study and the \( j \) level, and \( e_{ij} = \) residual error. A significant effect was declared at \( p<0.05 \) or there is a tendency when the \( p \)-value was between 0.05 and 0.10. Comparison among the experimental group was conducted with least square means and adjusted with Tukey’s test.

### RESULTS

The dose of EOs administration on productive performance, nutrient digestibility, cecal microbes, and serum metabolites of broiler chickens

The regression equations between the dose of EOs and broiler productive performance, nutrient digestibility, intestinal profile, and serum metabolites are presented in Table 4 and 5. The productive parameters (BWG and final BW) represent the productive performance, nutrient digestibility, cecal microbes, and serum metabolites. The dose of EOs administration on productive performance, nutrient digestibility, cecal microbes, and serum metabolites is shown in Table 4 and 5. The regression equations between the dose of EOs and broiler productive performance, nutrient digestibility, intestinal profile, and serum metabolites are presented in Table 4 and 5. The productive parameters (BWG and final BW) represent the productive performance, nutrient digestibility, cecal microbes, and serum metabolites.

### Table 2. Descriptive statistics of nutrient specifications used in meta-analysis

| Parameters | Unit     | n  | Mean   | SD    | Min   | Max   |
|------------|----------|----|--------|-------|-------|-------|
| Nutrient specification | Kcal/kg |    |        |       |       |       |
| Starter    |          |    |        |       |       |       |
| ME         | Kcal/kg  | 139| 3.017  | 99.9  | 2.796 | 3.300 |
| Crude protein | %     | 147| 22.08  | 1.20  | 18.77 | 27.68 |
| Ether extract | %    | 70 | 6.32   | 0.16  | 3.69  | 9.81  |
| Lysine     | %       | 117| 1.27   | 0.108 | 0.97  | 1.45  |
| Grower     |          |    |        |       |       |       |
| ME         | Kcal/kg  | 81 | 3.089  | 77.3  | 2.970 | 3.300 |
| Crude protein | %     | 89 | 20.74  | 1.27  | 19.00 | 26.16 |
| Ether extract | %    | 53 | 7.19   | 0.14  | 5.50  | 9.10  |
| Lysine     | %       | 61 | 1.16   | 0.099 | 0.97  | 1.39  |
| Finisher   |          |    |        |       |       |       |
| ME         | Kcal/kg  | 96 | 3.136  | 90.1  | 3.014 | 3.300 |
| Crude protein | %     | 96 | 19.46  | 1.45  | 17.00 | 24.64 |
| Ether extract | %    | 41 | 7.55   | 0.12  | 6.50  | 9.50  |
| Lysine     | %       | 76 | 1.10   | 0.10  | 0.90  | 1.33  |

SD, standard deviation; Max, maximum; Min, minimum; ME, metabolizable energy.
decrease (p<0.001) with increasing EOs doses except for feed intake that showed a quadratic model (p<0.001). Similarly, positive effects of EOs inclusion were also detected on increasing apparent DM, CP, and EE digestibility with a linear pattern (p<0.001) which may be a factor promoting the increase in BWG. Administration of EOs also increased carcass and gizzard and decreased abdominal fat percentage relative to BW (p<0.01) and some of the lymphoid organ weight such as the spleen, bursa fabricius (BF), and pancreas although the pattern of spleen and BF followed quadratic relationship (p<0.05). To a lesser extent, liver tended to show a quadratic pattern (p = 0.054).

Another beneficial effect of supplementing EOs in the broiler diet was shown by linearly increasing (p<0.001) *Lactobacillus* and suppressing *Escherichia coli* (*E. coli*) population in the cecum. The population of coliform also linearly increased (p = 0.03) although the effect was smaller. Villus height of the broiler intestine increased as the EOs doses increased (p<0.001) but the villus width showed a contrary result (p = 0.007). A different response was observed on crypt depth which was affected quadratically by EOs administration (p<0.05) while the height/depth criterion decreased linearly (p<0.05).

Responses of serum metabolites to dietary EOs were posi-

### Table 3. Descriptive statistics of the variables used in meta-analysis

| Parameters                          | Unit     | n   | Mean  | SD    | Min   | Max   |
|-------------------------------------|----------|-----|-------|-------|-------|-------|
| Production performance              |          |     |       |       |       |       |
| Age                                 | d        | 163 | 36.0  | 9.4   | 14.0  | 46.0  |
| Body weight                         | g        | 152 | 2,095 | 731.6 | 525.1 | 3,436 |
| Feed intake                         | g/period | 149 | 3,525 | 1,334.5 | 757.6 | 5,735 |
| Feed conversion ratio               | g feed/g gain | 149 | 1.69 | 0.21 | 0.71 | 2.14 |
| Body weight gain                    | g/bird/d | 152 | 56.48 | 13.73 | 25.01 | 83.90 |
| Average daily feed intake           | g/d      | 149 | 95.80 | 25.31 | 36.07 | 152.0 |
| Nutrient digestibility              |          |     |       |       |       |       |
| Dry matter                          | %        | 23  | 71.20 | 6.56  | 56.20 | 79.01 |
| Crude protein                       | %        | 30  | 72.45 | 10.51 | 45.30 | 86.50 |
| Ether extract                       | %        | 20  | 85.28 | 6.66  | 73.30 | 94.80 |
| Relative organ weight               |          |     |       |       |       |       |
| Carcass                             | % BW     | 34  | 71.37 | 0.922 | 59.48 | 78.85 |
| Breast meat                         | % BW     | 10  | 28.06 | 13.86 | 14.81 | 45.22 |
| Abdominal fat                       | % BW     | 23  | 1.76  | 0.62  | 0.66  | 2.83  |
| Gizzard                             | % BW     | 26  | 1.98  | 0.190 | 0.63  | 3.40  |
| Lymphoid organ weight               |          |     |       |       |       |       |
| Liver                               | % BW     | 57  | 2.43  | 0.088 | 1.73  | 5.25  |
| Spleen                              | % BW     | 44  | 0.20  | 0.35  | 0.08  | 1.79  |
| Bursa of Fabricius                  | % BW     | 31  | 0.19  | 0.03  | 0.10  | 0.27  |
| Pancreas                            | % BW     | 38  | 0.45  | 0.50  | 0.20  | 1.75  |
| Cecal microbes                      |          |     |       |       |       |       |
| *Escherichia coli*                  | Log CFU/g | 26  | 7.03  | 1.20  | 5.11  | 9.56  |
| *Lactobacillus*                     | Log CFU/g | 30  | 7.33  | 1.91  | 1.97  | 9.81  |
| *Clostridium*                       | Log CFU/g | 21  | 7.19  | 0.97  | 5.49  | 8.40  |
| Intestinal morphology               |          |     |       |       |       |       |
| Villus height                       | μm       | 29  | 858.0 | 465.1 | 286.0 | 1752 |
| Villus width                        | μm       | 14  | 148.7 | 87.7  | 80.5  | 404.4 |
| Crypt depth                         | μm       | 29  | 154.9 | 15.92 | 60.0  | 404.0 |
| Height/depth                        | μm       | 12  | 5.35  | 2.44  | 2.67  | 9.10  |
| Serum metabolites                   |          |     |       |       |       |       |
| IgG                                 | μg/mL    | 13  | 13.62 | 11.75 | 4.03  | 39.41 |
| Triglycerides                       | mg/dL    | 39  | 92.02 | 46.81 | 28.60 | 178.0 |
| Cholesterol                         | mg/dL    | 44  | 138.80| 80.82 | 42.00 | 443.8 |
| HDL                                 | mg/dL    | 18  | 78.42 | 25.21 | 50.20 | 139.9 |
| LDL                                 | mg/dL    | 15  | 79.35 | 77.80 | 33.80 | 273.0 |
| Glucose                             | mg/dL    | 31  | 249.9 | 5.32  | 189.3 | 320.2 |
| Albumin                             | mg/dL    | 15  | 2.16  | 0.81  | 0.92  | 3.70  |
| Protein                             | mg/dL    | 25  | 4.35  | 2.03  | 1.36  | 7.60  |
| Globulin                            | mg/dL    | 12  | 2.19  | 0.633 | 1.70  | 3.80  |

SD, standard deviation; Max, maximum; Min, minimum; IgG, immunoglobulin G; HDL, high density lipoprotein; LDL, low density lipoprotein.
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Table 4. Regression equations on the effect of essential oils dose (in mg/kg of diet) on production performance, nutrient digestibility, and organ weight of broiler chickens

| Response variables                        | n  | Model | Intercept | \( SE_{\text{Intercept}} \) | Slope | \( SE_{\text{Slope}} \) | p-value | RMSE  | AIC(1) | BIC(2) |
|------------------------------------------|----|-------|-----------|-----------------------------|-------|--------------------------|---------|-------|--------|--------|
| Production performance                   |    |       |           |                             |       |                          |         |       |        |        |
| Body weight (g)                          | 152| L     | 2,065     | 110.5                       | 0.1668| 0.1791                   | <0.0001 | 267.79| 2,267  | 2,283  |
| Feed intake (g/period)                   | 149| Q     | 3,482     | 207.6                       | -0.2197| 0.3095                   | <0.0001 | 445.54| 2,392  | 2,396  |
| Feed conversion ratio                    | 149| L     | 1.67      | 0.031                       | -0.0002| 0.00004                  | <0.0001 | 0.06  | -212   | -208   |
| Body weight gain (g/bird·d)              | 152| L     | 55.7      | 2.03                        | 0.004 | 0.004                    | <0.0001 | 5.24  | 1,082  | 1,104  |
| ADFI (g/bird·d)                          | 149| L     | 94.5      | 3.90                        | -0.006| 0.006                    | <0.0001 | 8.78  | 1,225  | 1,228  |
| Nutrient digestibility (%)               |    |       |           |                             |       |                          |         |       |        |        |
| Dry matter                               | 23 | L     | 71.31     | 2.108                       | 0.0019| 0.0049                   | <0.0001 | 2.83  | 141    | 145    |
| Crude protein                            | 30 | L     | 73.71     | 2.505                       | 0.0048| 0.0068                   | <0.0001 | 5.58  | 215    | 218    |
| Ether extract                            | 20 | L     | 85.92     | 2.369                       | 0.0058| 0.0051                   | <0.0001 | 3.83  | 129    | 133    |
| Relative organ weight (% BW)             |    |       |           |                             |       |                          |         |       |        |        |
| Carcass                                  | 34 | L     | 60.67     | 2.078                       | 0.004 | 0.003                    | <0.0001 | 1.45  | 167    | 170    |
| Breast meat                              | 10 | L     | 23.51     | 7.651                       | 0.006 | 0.002                    | 0.054   | 0.68  | 55     | 58.6   |
| Abdominal fat                            | 23 | L     | 1.88      | 0.203                       | -0.0001| 0.0003                   | <0.0001 | 0.17  | 31     | 34.4   |
| Gizzard                                  | 26 | Q     | 3.00      | 0.711                       | 0.000573| 0.0012                   | 0.04    | 0.31  | 91     | 94.2   |
| Lymphoid organ weight (% BW)             |    |       |           |                             |       |                          |         |       |        |        |
| Liver                                    | 57 | Q     | 5.52      | 2.622                       | 0.001048| 0.001163                 | 0.067   | 0.51  | 235    | 239    |
| Spleen                                   | 44 | Q     | 0.25      | 0.108                       | -0.00001| 0.000035                 | 0.057   | 0.01  | -98    | -94.4  |
| Bursa of Fabricius                       | 31 | Q     | 0.19      | 0.012                       | -2.73E-6| 0.000094                 | <0.0001 | 0.02  | -73    | -69.3  |
| Pancreas                                 | 38 | L     | 0.58      | 0.156                       | 0.0001| 0.00004                  | 0.006   | 0.03  | -70    | -66    |

SE, standard error; RMSE, root mean square error; AIC, akaike information criterion; BIC, bayesian information criterion; L, linear; Q, quadratic; ADFI, average daily feed intake; BW, body weight.

1) AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better).
2) BIC is an estimator of a probability of a model being true (smaller is better).

tive, as the EOs linearly reduced low density lipoprotein (LDL) concentration (p<0.01) and concomitantly increased high density lipoprotein (HDL), glucose, protein, and globulin concentrations at a linear pattern (p<0.01). The concentration of triglycerides and cholesterol linearly and quadratically decreased in response to elevating the dose of EOs (p<0.01). There was no effect of EOs inclusion on immunoglobulin metabolite (IgG) as shown in Table 5 (p = 0.169).

Comparison of the effectiveness of the type of EOs and AGP on broiler performance

The effectiveness of EOs to replace the use of AGP in terms of improving broiler performance was examined and the comparison results are presented in Table 6 and 7. There was marginal evidence on the effect of EOs administration on daily weight gain (BWG) and final BW of broiler chickens whereas EOB 1 increased (p<0.05) the BWG and final BW (p<0.05) by 8.52% and 11.17%, respectively compared to those of Con and AB groups. The FCR significantly decreased (p<0.001) by employing all types of EOs (Thy-BC, Car-BC, Cin-BC, Men-BC, EOB 1, and EOB 2) whereas the Car-BC showed the largest reduction (7.14%) in comparison with the control group. There was no effect (p>0.05) of AB on FCR. The inclusion of EOs in this study did not influence (p = 0.956) ADFI and cumulative FI (p = 0.967). Type of EOs also failed to increase the digestibility of DM, CP, and EE compared with the use of AGP and that did not receive any additive (p>0.10). Cin-BC treatment tended to increase (p = 0.067) carcass weight by 5.67% while Thy-BC group tended to increase (p = 0.071) relative weight of liver (Table 7).

The population of E. coli significantly reduced (p<0.01) with Cin-BC and EOB 1 treatments by 8.88% and 7.76%, respectively while the population of Coliform and Lactobacillus did not differ among the treatment groups (p>0.10). There was no difference in the intestinal morphology of broiler chickens in response to the administration of AGP or type of EOs (p>0.10). Furthermore, due to relatively large variation of the data of immunoglobulin concentration and serum metabolites parameters, most of these parameters including the concentration of IgG, triglycerides, cholesterol, HDL, and glucose did not change with the application of EOs in comparison to AB and control groups (p>0.10).
Table 5. Regression equations on the effect of essential oils dose (in mg/kg of diet) on cecal microbes, intestinal morphology, and serum metabolites profile of broiler chickens

| Response variables | n  | Model  | Variable estimates | Model estimates | p-value | RMSE | AIC | BIC |
|-------------------|----|--------|--------------------|----------------|---------|------|-----|-----|
|                   |    | Intercept | SE Intercept | Slope | SE Slope |       |       |     |     |
| Cecal microbes (Log CFU/g) |    |        |           |       |         |       |       |     |     |
| Escherichia coli   | 26 | L      | 6.98      | 0.440 | -0.0002 | 0.0006 | <0.0001 | 0.46 | 76  |
| Lactobacillus      | 30 | L      | 7.10      | 0.645 | 0.0010  | 0.0003 | <0.0001 | 0.26 | 74  |
| Coliform           | 21 | L      | 5.42      | 1.648 | 0.0003  | 0.0006 | 0.03  | 0.37 | 57  |
| Intestinal morphology (μm) |    |        |           |       |         |       |       |     |     |
| Villus height      | 29 | L      | 920.1     | 163.03| 0.198  | 0.102  | 0.0005 | 88.13 | 375 |
| Villus width       | 14 | L      | 129.9     | 19.98 | -0.018  | 0.034  | 0.0074 | 15.26 | 127 |
| Crypt depth        | 29 | Q      | 170.5     | 30.46 | -0.1023 | 0.09882 | 0.0006 | 23.12 | 313 |
| Height/depth       | 12 | L      | 5.6       | 1.25  | -0.0002 | 0.002  | 0.0211 | 1.55  | 63  |
| Serum metabolites  |    |        |           |       |         |       |       |     |     |
| IgG (μg/mL)        | 13 | L      | 10.99     | 6.107 | 0.013   | 0.004  | 0.169 | 2.43  | 83  |
| Triglycerides (mg/dL) | 39  | Q      | 87.68     | 14.056| 0.01704 | 0.02387 | 0.001 | 7.50  | 334 |
| Cholesterol (mg/dL) | 44 | Q      | 131.05    | 22.050| -0.04666| 0.04605 | 0.000209| 0.0001 | <0.0001 |
| HDL (mg/dL)        | 18 | L      | 75.88     | 13.990| 0.019   | 0.009  | 0.0056 | 5.65  | 139 |
| LDL (mg/dL)        | 15 | L      | 46.16     | 3.489 | -0.022  | 0.007  | 0.0009 | 3.56  | 97  |
| Glucose (mg/dL)    | 28 | L      | 240.18    | 11.120| 0.050   | 0.032  | <0.0001 | 14.92 | 251 |
| Albumin (mg/dL)    | 15 | Q      | 1.62      | 0.253 | 0.00253 | 0.0014 | 0.0032 | 0.18  | 47  |
| Protein (mg/dL)    | 25 | L      | 4.18      | 0.616 | 0.001   | 0.001  | 0.0011 | 0.55  | 76  |
| Globulin (mg/dL)   | 12 | L      | 1.93      | 0.329 | 0.001   | 0.001  | 0.0009 | 0.68  | 41  |

SE, standard error; RMSE, root mean square error; AIC, akaike information criterion; BIC, bayesian information criterion; CFU, colony forming unit; IgG, Immunoglobulin G; HDL, high density lipoprotein; LDL, low density lipoprotein.

1) AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better).
2) BIC is an estimator of a probability of a model being true (smaller is better).

Table 6. Effect of antibiotics and EOs administration on production performance, nutrient digestibility, and organ weight of broiler chickens

| Response variables | Unit | n  | Con | AB | Type of EOs bioactive compounds | SEM | p-value |
|-------------------|------|----|-----|----|---------------------------------|-----|---------|
|                   |      |    |     |    | Thy-BC, Car-BC, Cin-BC, Men-BC, EOB 1, EOB 2 |     |         |
| Production performance |      |    |     |    |                                 |     |         |
| Body weight       | g    | 152 | 2.042 | 2.075 |                                 |     |         |
| Feed Intake       | g    | 149 | 3.476 | 3.487 |                                 |     |         |
| Feed conversion ratio | g/ feed/g gain | 149 | 1.68 | 1.66 | Thy-BC, Car-BC, Cin-BC, Men-BC, EOB 1, EOB 2 |     |         |
| Body weight gain  | g/ bird/d | 152 | 55.05 | 55.71 |                                 |     |         |
| Nutrient digestibility |      |    |     |    |                                 |     |         |
| Dry matter        | %   | 23  | 70.85 | 71.79 |                                 |     |         |
| Crude protein     | %   | 30  | 72.41 | 74.46 |                                 |     |         |
| Ether extract     | %   | 20  | 84.98 | 83.86 |                                 |     |         |
| Relative organ weight |    |    |     |    |                                 |     |         |
| Carcass           | % BW| 34  | 69.36 | 69.88 |                                 |     |         |
| Abdominal fat     | % BW| 23  | 1.89  | 2.13 |                                 |     |         |
| Lymphoid organ weight |    |    |     |    |                                 |     |         |
| Liver             | % BW| 57  | 2.32  | 2.23 |                                 |     |         |
| Spleen            | % BW| 44  | 0.24  | 0.26 |                                 |     |         |
| Bursa of Fabricius| % BW| 31  | 0.19  | 0.19 |                                 |     |         |
| Pancreas          | % BW| 38  | 0.58  | 0.57 |                                 |     |         |

EOs, essential oils; SEM, standard error of means; BW, body weight.

1) Con, control; AB, antibiotics; Thy-BC, thymol-based compound; Car-BC, carvacrol-based compound; Cin-BC, cinnamaldehyde-based compound; Men-BC, menthol-based compound; EOB 1, essential oils blend containing one of more of thymol, carvacrol, and cinnamaldehyde compounds; EOB 2, essential oils blend based on terpenoids group (linalool, geraniol, thujanol, borneol, menthol, citronellol, terpineol).

Value with different letters differ at p < 0.05.
However, a positive effect was observed on significantly reduced (p<0.05) LDL concentration by 14.55% and 17.86%, respectively using AB and Car-BC when compared to the control group. Globulin concentration increased (p<0.05) by 39.43% with Cin-BC treatment while this treatment also tended to increase (p<0.1) the albumin and protein concentration of the serum metabolite.

**DISCUSSION**

Empirical works evaluating the efficacy of EOs bioactive compounds on broiler performance are abundant, which of those have been regularly reviewed [3,4,17]. However, no study so far has attempted to quantitatively summarize the effect of EOs on broiler performance by employing a meta-analysis approach. An increase in final BW and a concomitant effect of EOs on broiler performance by employing a meta-analysis. Such an effect can be attributed to the improved growth performance of the broiler chickens, which is well-documented in the literature [18-20]. The positive impact of EOs on BW is likely due to their stimulatory effects on feed intake and nutrient absorption, which are well-documented in the literature [21-23]. The ability of EOs to stimulate feed intake and nutrient absorption is likely due to their stimulatory effects on digestive enzymes, which are well-documented in the literature [24-26].

However, a significant quadratic pattern on BW and FCR when using sweetgum (Liquidambar orientalis Mill.) leaves that contained g- Terpinen-4-ol and g-terpinene as major components, with an optimum dose of 80 mg/kg. In regard to feed intake, available literatures suggested that EOs effect can be either stimulate or suppress feed palatability, depending on their level, types, and bird age [28-30].

Inconclusive findings among previous studies underlying the mechanism on how dietary EOs could improve broiler performance are at least partially overcome in this current meta-analysis. Such an effect can be attributed to the increasing nutrient digestibility, gizzard weight (capacity), and villus height as shown in the relationship between the dose of EOs and response variables. Previously, similar results were reported that EOs inclusion improved apparent ileal digestibility of nutrients at 21, 35, and 42 d of age [9]. Little is known about the effect of EOs on enzyme secretion at the early age of broiler because, to our knowledge, there is no study examining nutrient digestibility at the age of less than 14 d in regard to EOs incorporated-diet. Thus, it was speculated that EOs may show a lack of ability to promote enzyme secretion in the pre-starter or starter phase of broiler because in this period, indigenous enzyme secretion is very low [31].

It has been recognized that increasing nutrient digestibility primarily occurred on adult birds, apparently due to the improvement of small intestine morphology and microbial balance, and possibly because of the stimulating effect on an
increase in bile acids and digestive and pancreatic enzymes secretion [30,32].

Furthermore, most studies supported the available hypothesis that the effect on enzyme was more pronounced in adult birds. For instance, there was a linear increase in trypsin, lipase, and protease activities on the 24-d broiler intestine fed with EOs containing-phytogenic [27]. Similarly, higher activity and production of the pancreatic and digestive enzyme were exhibited on birds received thymol [32] or oregano EOs [30] while Masouri et al [33] also confirmed an increase in digestive juices which has antimicrobial effects by supplementing EOs. In addition to the positive effects aforementioned, it is primarily important to further investigate a factor related to enzyme secretion enhancement by supplementing EOs because few studies also reported absence effect on that [34] which may be due to difference in bioactive compounds, animal age, hygiene, diet type, and also environmental factors [27].

As confirmed in this study, the abundance of *Lactobacillus* increased while the population of *E. coli* decreased, whereas this result is in line with previous studies that reported similar results [26,35]. It has been widely known that EOs bioactive compounds are broad-spectrum antibacterial, antiviral, and antifungal. Using 45 EOs, Chao et al [36] found that all of these compounds effectively inhibited eight genera of bacteria with different degrees of inhibitory effects. Some were more sensitive to either gram-negative or gram-positive bacteria and some others were effective for both types of bacteria, depending on the variation in chemical compositions of the EOs. Interestingly, the relationship between major and trace components of the EOs was also varied among others which can be synergistic, additive, or antagonistic [37,38].

It is generally accepted that there is more than one mode of action regarding their antibacterial role although the specific mechanism is not clear. In regard to their mode of action, EOs bioactivity can be recognized from their multiple benefits such as antimicrobial, antioxidant, immunomodulator, and anti-inflammatory properties [3]. Available explanations suggested that the antimicrobial effect is largely due to the hydrophobicity characteristics which allow the substances to disturb the permeability of the cell wall of bacteria and its mitochondria. EOs components such as carvacrol, eugenol (2-methoxy-4-(2-propenyl)phenol), and thymol, for example, can depolarize the cytoplasmic membrane of gram-negative bacteria by disintegrating the outer membrane of targeted bacteria [39]. Alteration of intestinal flora is one of the important roles of EOs to act as growth-promoting additive. More recently, microbiome study in the cecal part of broiler confirmed an enrichment of phyla Bacteroidetes and genera Alistipes which play at promoting animal growth by supplementing EOs [40].

In addition to the antimicrobial effect, they are also able to stimulate digestive enzyme secretion and activity and increase bile synthesis that can positively increase the digestibility of nutrients. Moreover, many of EOs constituents showed to improve antioxidant status especially EOs extracted from aromatic plants. Their antioxidant activity was also related to the ability of EOs to reduce cell proliferation and act as anti-inflammatory properties. It was reported that EOs could scavenge reactive oxygen species from bacterial phagocytosis process, resulting in the reduction of tissue oxidative damage and inflammation [8].

An increase in villus height in response to dietary EOs was supported by other studies [10,41] but it was not sufficient to elucidate the mode of action. Nevertheless, Hamedi et al [42] reported there was no effect on villus height and crypt depth by incorporating EOs from peppermint and thyme. Because there is limited information on that, several authors suggested that it might be a result of toxins reducing effect in the gastrointestinal tract because of the modulation of microflora [43]. Also, EOs can enhance the mature enterocytes that can improve absorption capacity due to an increase in the villus height and decrease the crypt depth [26].

EOs also exhibited a positive effect on the organ composition of broiler chickens. This is consistent with numerous studies using different EOs sources [9,25,26]. It is well explained that active components of EOs such as carvacrol, thymol, menthol, and p-cymene were involved in the lipids metabolism, particularly on serum cholesterol [44]. It was found that phenol and flavonoids compounds of EOs reduced rate-limiting enzyme involved in cholesterol synthesis, 3 hydroxy-3-methylglutaryl coenzyme A reductase because it is a key enzyme for cholesterol production [45]. As a result, not only less fat accumulation and more carcass portion, but also a positive correlation to the decreased of cholesterol, triglycerides, and LDL concentrations of serum metabolites were produced in this study. At the same time, blood protein, globulin, glucose, and albumin also increased. These were in agreement with several experiments in which dietary EOs extracted from a variety of plants reduced cholesterol levels [46], and increased blood albumin, globulin, and protein [47,48]. EOs supplementation also affected lymphoid organ weight. Previous studies also demonstrated that EOs blend increased BF and thymus [26,49] but in contrast with Rahimi et al [50]. The effect of EOs on immune function of broiler is apparently weaker compared to its effects as a gut modulator, enzyme stimulator, and growth promoter. This is supported that by the fact that the immunoglobulin concentration of IgG was not affected by the EOs administration in this study.

To better understand which type of EOs affected the broiler performance, analysis of moderator variables that may affect the magnitude of EOs was conducted by grouping the EOs based on references evaluating the degree of antimicrobial
effects. Accordingly, this study found that EOs blend containing more than two combination of thymol, carvacrol, cinnamaldehyde, and menthol had a greater effect to increase ADG and final BW of broiler chickens than other type of EOs although the effect was not substantial. In agreement with this study, some previous reports showed that combination of thymol and cinnamaldehyde [36,51] or thymol, carvacrol, and cinnamaldehyde [52,53] had greater antimicrobial effect than antibiotics and significantly increased broiler productive performance. In addition, all types of EOs bioactive compounds also significantly reduce FCR compared to the control and antibiotics groups. This result was also in agreement with previous experiments [54,55] who found that the main effect of antibiotics and EOs was more significant on lowering FCR where the effect on BWG and final BW was rather low. Likewise, in this study EOs promoted higher population of Lactobacillus and lower population of E. coli than antibiotics which also reasonably increase feed efficiency. This finding suggested that by using EOs, more economic profit seems to be earned as feed conversion efficiency is of the most important indicator in the broiler industry, in the condition when the relative value of EOs products are similar with AGP. Accordingly, investigations on the economic aspect of various conditions are demanding.

CONCLUSION

The present meta-analysis confirms that there are positive effects on broiler productive performance as the inclusion levels of EOs increase, regardless of type of components used. Across all studies, the growth-promoting effect of EOs is strongly related to their strategic role in many metabolic pathways as antimicrobial, antioxidant, and anti-inflammatory agents resulting in an increase in nutrient digestibility, carcass percentage, gut integrity, and metabolites profile. Most of the parameters outcome show linear patterns, indicating doses of EOs given are effective to facilitate better growth performance of broiler chickens. This study also demonstrated that in comparison to antibiotics, type of EOs have variable results for BWG, final BW, and FCR. Among EOs bioactive compounds, EO-Blend of thymol, carvacrol, and cinnamaldehyde showed higher efficacy in increasing productive performance and feed efficiency. These points confirm that EOs are suitable to be used as growth promoters and their economical benefit may be promising.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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