Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
32 (4): 475-492, 2026
DOI: 10.9775/kvfd.2026.36578

Review Article

Efficacy of Essential Oil-based Interventions Against Avian Coccidiosis: A Meta-analysis

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How to cite this article?
Alshammari A: Efficacy of Essential Oil-based Interventions Against Avian Coccidiosis: A Meta-analysis. *Kafkas Univ Vet Fak Derg*, 32 (4): 475-492, 2026.
DOI: 10.9775/kvfd.2026.36578

Article ID: KVFD-2026-36578
Received: 12.03.2026
Accepted: 19.07.2026
Published Online: 28.07.2026

Abstract

Avian coccidiosis remains one of the most important parasitic diseases of poultry worldwide. Resistance to conventional anticoccidials and limitations of vaccination programs have stimulated the search for natural alternatives. Essential oils (EOs) are complex mixtures of bioactive terpenoids and phenolics. They possess antimicrobial, anti-inflammatory, and immunomodulatory activities that may disrupt *Eimeria* infection and enhance host resilience. This study aimed to quantify the efficacy of essential oil-based interventions, including individual oils, essential oil blends, and essential oil-containing multicomponent formulations, in poultry experimentally challenged with *Eimeria* spp. through a meta-analysis conducted according to PRISMA 2020 guidelines. For this, literature searches across major databases identified experimental trials. Eighteen eligible studies were included, reporting outcomes on BWG, feed intake, FCR, mortality, fecal oocyst counts, and serum total protein. EO supplementation was associated with a small, statistically non-significant increase in body weight gain (MD +8.08 g, 95% CI –0.08 to 16.24) and a modest improvement in feed conversion ratio (MD –0.13, 95% CI –0.24 to –0.01). Mortality was reduced (RR 0.49, 95% CI 0.27-0.89), and oocyst shedding decreased (MD –18.99, 95% CI –37.91 to –0.08). No consistent effects were observed for feed intake or serum total protein. Overall, essential oils may offer anticoccidial benefits, particularly for feed efficiency, mortality, and oocyst shedding, although their effect on body weight gain remains uncertain.

Keywords: *Eimeria*, Essential oils, Meta-analysis, Natural anticoccidials, Oocyst shedding, Poultry

Introduction

Avian coccidiosis is an intestinal disease caused by protozoan parasites of the genus *Eimeria*. It remains one of the most pervasive and costly challenges in global poultry production [1,2]. Infected birds suffer mucosal damage leading to diarrhea, poor nutrient absorption, reduced growth and egg production, and, in severe outbreaks, high morbidity and mortality [3]. The disease also predisposes chickens to secondary bacterial infections (e.g., *Clostridium perfringens*, *Salmonella*, *Campylobacter)* [1]. Coccidiosis is widely recognized as the parasitic disease with the highest economic impact on the poultry industry. Coccidiosis causes multibillion-dollar economic losses to the global poultry industry annually [4]. These losses arise from reduced feed efficiency, slower weight gain, increased feed and medication costs, and carcass condemnation [5].

Current control of coccidiosis relies heavily on prophylactic use of anticoccidial drugs (ionophores such as monensin, salinomycin, and synthetic chemicals like diclazuril) and live vaccines. These strategies can reduce disease severity, but each has significant drawbacks [6]. The extensive use of ionophores and chemical coccidiostats over decades has led to widespread development of drug-resistant *Eimeria* strains [1,7]. In practice, many flocks now harbor parasites with reduced sensitivity to one or more anticoccidials [8]. Moreover, drug residues in meat raise consumer safety and regulatory concerns [9]. In the European Union, for example, routine in-feed anticoccidials are banned except by veterinary prescription, reflecting public health and welfare pressures [7]. Anticoccidial vaccines (live oocyst vaccines) offer a drug-free option but also have limitations: they typically protect against only specific *Eimeria* species, require high cost and repeated administration, and can fail if production parameters or strain variations intervene [10]. Importantly, both therapeutic and vaccination programs are expensive, and public sentiment increasingly favors “chemical-free” poultry products. Even where vaccines are used, their high-cost limits use mainly to breeder or parent stock rather than large commercial broiler operations [7]. In short, traditional control measures are valuable but imperfect, and producers and consumers alike are seeking safer, sustainable alternatives [11].

Against this background, plant-derived essential oils (EOs) have attracted growing interest as natural anti-coccidials. EOs are complex mixtures of terpenoids, phenolics, and other phytochemicals (for example, thymol and carvacrol in *Lamiaceae* oils, allicin and ajoene in garlic oil, eugenol in clove oil, cinnamaldehyde in cinnamon oil) that have antimicrobial, anti-inflammatory, and immunomodulatory properties. EOs are generally recognized as safe for food; they are viewed as consumer-friendly alternatives [5,12,13]. EO supplementation can exert anticoccidial effects *in vivo*. They can result in improvements in body weight gain and feed conversion, coupled with reductions in oocyst shedding and lesion scores [14]. Experimental work has also demonstrated that phenolic EO components such as thymol, carvacrol, and eugenol can disrupt parasite sporulation and compromise oocyst wall integrity, supporting a direct anticoccidial mechanism [15,16]. In addition to their antiparasitic properties, EOs can enhance gut health through improved villus morphology, stabilization of intestinal barrier function, and modulation of gut microbiota. These effects may improve nutrient utilization and increase resilience to infection. The dietary supplementation with essential oils also holds promise as a natural strategy for mitigating the effects of coccidiosis in poultry [17]. Nevertheless, the variability in outcomes highlights the need for quantitative synthesis and critical evaluation. A meta-analytical approach can integrate the data from many studies to estimate overall effect sizes of EO supplementation on key outcomes and to identify moderators of efficacy. Such analysis is timely and necessary because the inconsistent literature makes it hard to draw general conclusions about whether and how much EOs can replace or supplement anticoccidials in practice.

Previous reviews have primarily provided narrative summaries of the anticoccidial properties, potential mechanisms, and practical applications of essential oils and other phytogenic compounds in poultry [5-7,14]. Although these reviews have highlighted promising findings, they have not quantitatively estimated the overall magnitude and consistency of essential oil effects across controlled *Eimeria*-challenge studies. Consequently, uncertainty remains regarding whether the reported benefits are reproducible across different essential oil preparations, challenge models, and measured outcomes. The present meta-analysis addresses this gap by pooling comparative data for growth performance, feed efficiency, mortality, oocyst shedding, and serum total protein, while also evaluating heterogeneity, publication bias, and the influence of individual studies.

In this study, we conducted a comprehensive meta-analysis of controlled experiments in which poultry were challenged with *Eimeria* spp. and fed essential oils or EO-containing botanicals. We examined the impact of EO interventions on parameters relevant to coccidiosis: body weight gain, feed intake, feed conversion ratio, mortality, fecal oocyst counts (OPG), and serum total protein levels. By pooling data globally, we aimed to overcome the inconsistencies of individual trials and derive robust estimates of EO efficacy. The outcomes are of high practical importance. The weight gain, feed efficiency and mortality determine production performance and profitability, while OPG and blood protein reflect parasite burden and bird health. This meta-analysis provides a systematic synthesis of available evidence on the effects of essential oil supplementation in *Eimeria*-challenged poultry, addressing variability across individual studies and offering a more integrated assessment of their efficacy.

Material and Methods

Protocol and Reporting Standards

The meta-analysis was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 statement [18]. The review protocol was not prospectively registered in PROSPERO or another systematic review registry. The eligibility criteria, literature search strategy, data extraction procedures, and statistical methods used in the review are described in the following sections to support transparency and reproducibility.

Literature Search Strategy

A comprehensive literature search was performed in PubMed, Web of Science Core Collection, Scopus, CAB Abstracts, and Google Scholar from database inception through June 2025. The complete PubMed search strategy was: ((“essential oil”[Title/Abstract] OR “essential oils”[Title/Abstract] OR thymol[Title/Abstract] OR carvacrol[Title/Abstract] OR eugenol[Title/Abstract] OR cinnamaldehyde[Title/Abstract] OR oregano[Title/Abstract] OR thyme[Title/Abstract] OR garlic[Title/Abstract] OR clove[Title/Abstract]) AND *(Eimeria* [Title/Abstract] OR coccidia[Title/Abstract] OR coccidiosis[Title/Abstract]) AND (poultry[Title/Abstract] OR chicken*[Title/Abstract] OR broiler*[Title/Abstract] OR hen*[Title/Abstract] OR turkey*[Title/Abstract] OR quail*[Title/Abstract])). This strategy was adapted to the indexing systems, field tags, and Boolean syntax of the other databases. No language restrictions were applied. Reference lists of the included studies and relevant review articles were also screened to identify additional eligible records.

All records were merged, and duplicate citations were identified and removed manually to yield a unique set of references. The unique records were screened by title and abstract, excluding clearly irrelevant studies. Full texts were retrieved for the remaining candidates and assessed for eligibility. All search activities and screening decisions were documented to permit reproduction.

Eligibility Criteria

Studies were eligible if they were original controlled *in vivo* trials conducted in domestic poultry experimentally challenged with *Eimeria* spp. Eligible interventions included individual essential oils, blends composed of multiple essential oils or their defined bioactive constituents, and multicomponent formulations in which one or more essential oils were explicitly identified as active ingredients. Multicomponent formulations containing essential oils together with organic acids, vitamins, minerals, tannins, or other phytogenic substances were retained because such formulations are commonly evaluated as integrated feed-based interventions in poultry production. The effects of these interventions were interpreted as those of the complete essential oil-based formulation rather than as effects attributable exclusively to the essential oil component.

Eligible studies were required to include an *Eimeria*-challenged control group that did not receive the tested intervention and to report numerical data for at least one of the following outcomes: body weight gain or final body weight, feed intake, feed conversion ratio, mortality, fecal oocyst count, or serum total protein. Reviews, observational studies, uncontrolled field surveys, *in vitro*-only studies, conference abstracts without sufficient data, and studies without an experimentally described *Eimeria* challenge were excluded. Studies were also excluded when the essential oil component was not identified, its administration or dose was not adequately described, no appropriate challenged control group was available, or relevant quantitative data could not be extracted.

Data Extraction and Variables Collected

Data from each included study were extracted manually by using a standardized form. Interventions were classified during data extraction as: (i) individual essential oils, (ii) blends containing only essential oils or their defined constituents, or (iii) essential oil-containing multicomponent formulations that also included other active substances. This classification was used to distinguish effects attributable to essential oil-only interventions from formulation-level effects that could also reflect the contribution of accompanying active components. For each trial, the following variables were recorded: first author, year of publication, country of origin, poultry type and breed/strain, *Eimeria* species used for challenge, type and source of essential oil (including plant name and chemical composition if reported), and dietary dose of EO (e.g. mg/kg feed). The primary outcomes and any additional reported endpoints were also captured. In particular, for each reported outcome, we collected the treatment and control group means (or proportions, for mortality) along with measures of variability (standard deviation [SD] or standard error [SE]) and the sample size (n) for each group. The six target outcomes (body weight gain, feed intake, feed conversion ratio, mortality, oocyst count, total protein) were extracted along with any other performance or health variables reported. Records of each study's data were checked for completeness and correctness by cross-referencing tables, text, and supplementary materials. Studies for which key quantitative data could not be obtained were not included in the meta-analysis.

Statistical Analysis

Meta-analyses were performed using R software version 4.4.1 with the meta and metafor packages. A random-effects model was fitted for each outcome because clinical and methodological variability was expected across studies. Between-study variance (τ^2) was estimated using the restricted maximum-likelihood (REML) method for all analyses. Continuous outcomes were summarized as mean differences (MDs) with 95% confidence intervals when measurements were reported in comparable units. Dichotomous mortality data were summarized as risk ratios (RRs) with 95% confidence intervals. Study-specific estimates were weighted using inverse-variance weighting.

Heterogeneity was assessed using Cochran's Q test and quantified using the I^2 statistic. Because I^2 estimates may be imprecise when few studies are available, they were interpreted together with τ^2, prediction intervals, and the clinical and methodological diversity among studies. The primary analyses included all eligible essential oil-based interventions. A separate quantitative subgroup analysis according to intervention composition was considered; however, it was not performed because only a small number of multicomponent formulations contributed to each outcome and their accompanying active ingredients differed substantially. Under these conditions, separate pooled estimates would have been statistically unstable and potentially misleading. Therefore, multicomponent formulations were additionally examined through a separate descriptive comparison, and their effects were interpreted at the formulation level rather than as isolated essential oil effects. Statistical significance of pooled effects was taken at $\alpha=0.05$ (two-sided). To evaluate the potential influence of small-study effects and publication bias, we applied visual and statistical methods. Funnel plots were generated for each outcome to inspect symmetry. Egger's regression test and Begg's rank-correlation test were used as exploratory assessments of funnel-plot asymmetry. Because these tests may have low power and produce unstable results when based on a small number of studies, their findings were interpreted cautiously and were not considered definitive evidence of publication bias. In addition, the trim-and-fill method was applied to estimate the number of potentially missing studies and to adjust the pooled effect sizes if necessary.

Additional analyses were conducted to probe the robustness of results. Influence diagnostics were computed using metafor functions: externally standardized residuals, DFFITS, Cook's distance, covariance ratios, and hat values were examined for outlying or influential studies [19]. A leave-one-out sensitivity analysis was performed by iteratively removing each study and recomputing the pooled effect [20]. Baujat plots were generated to identify studies that contributed disproportionately to overall variability. In a Baujat plot, each study's contribution to Cochran's Q is plotted against its influence on the pooled estimate, facilitating identification of outliers [21]. Finally, cumulative meta-analyses (adding studies in chronological order of publication) were performed to illustrate how the overall effect estimate evolved as evidence accumulated. For cumulative analyses, trials were ordered by publication year, and pooled effects were recomputed after each study addition [22].

All statistical analyses were conducted in R using functions from the metafor package, including rma() for random-effects model fitting and forest(), funnel(), influence(), baujat(), and cumul() for graphical and sensitivity analyses. The REML estimator was used consistently to estimate between-study variance for all continuous and dichotomous outcomes.

Data Presentation

Results of the meta-analyses were summarized in tables and figures. A summary table was prepared showing the pooled effect size (MD, SMD, or RR) and 95% confidence interval for each outcome. Graphical displays included forest plots (*Fig. 2, Fig. 3*) and funnel plots (*Fig. 4*). Forest plots displayed the study-specific effect estimates and 95% confidence intervals together with the pooled random-effects estimate obtained using the REML estimator. Key outcomes with sufficient studies were also depicted with funnel plots to visualize publication bias. Additional plots were generated to aid interpretation: Baujat plots (*Fig. 6*), influence plots (*Fig. 7, Fig. 8, Fig. 9, Fig. 10, Fig. 11, Fig. 12*) for outliers, and cumulative forest plots (*Fig. 5*) for evidence accumulation. The PRISMA flow diagram was included as *Fig. 1* (based on the recorded screening data).

Results

Literature Search and Study Selection

The systematic search across five databases (PubMed, Web of Science, Scopus, CAB Abstracts, and Google Scholar) yielded a total of 1,123 records. Following the removal of duplicates, 917 unique studies remained for screening. Title and abstract screening excluded 851 records as they did not meet the inclusion criteria (e.g., non-experimental design, absence of *Eimeria* challenge, use of non-poultry species, or interventions not involving essential oils). The remaining 76 full-text articles were assessed for eligibility. Of these, 58 studies were excluded after detailed review, most commonly due to incomplete or missing quantitative outcome data, combined interventions in which the effect of essential oils could not be isolated, or a lack of relevant outcomes. Ultimately, 18 experimental trials fulfilled all eligibility criteria and were included in the meta-analysis. These trials provided extractable data on at least one of the six pre-specified outcomes: body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), mortality, oocysts per gram (OPG), and serum total protein. The process of study identification, screening, exclusion, and inclusion is illustrated in the PRISMA flow diagram (*Fig. 1*), which provides a transparent overview of study selection and reasons for exclusion.

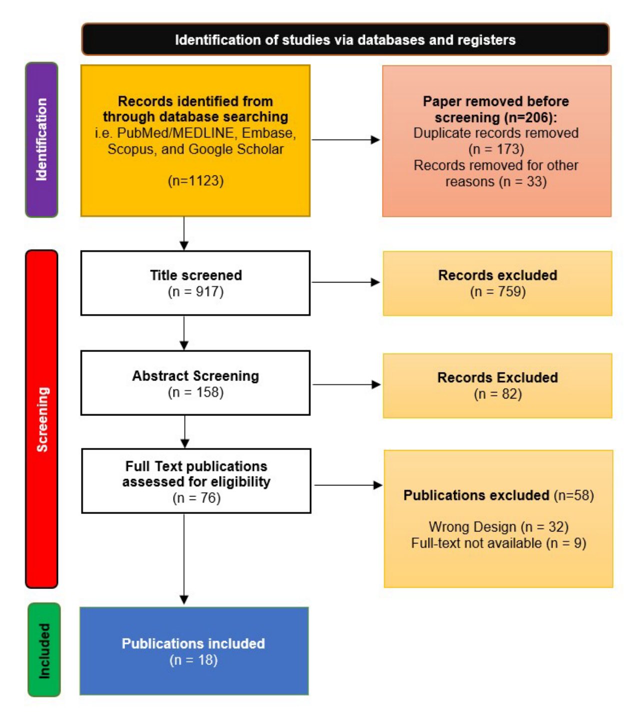

Fig 1. PRISMA Flow Chart in accordance with PRISMA 2020 statement

Study Characteristics

A total of 18 experimental trials fulfilled the eligibility criteria and were included in the meta-analysis (*Table 1*). All studies were conducted in poultry under controlled *Eimeria*-challenge conditions and reported at least one of the six prespecified outcomes: body weight gain, feed intake, feed conversion ratio, mortality, oocysts per gram of feces, or serum total protein. The interventions were classified as individual essential oils, essential oil-

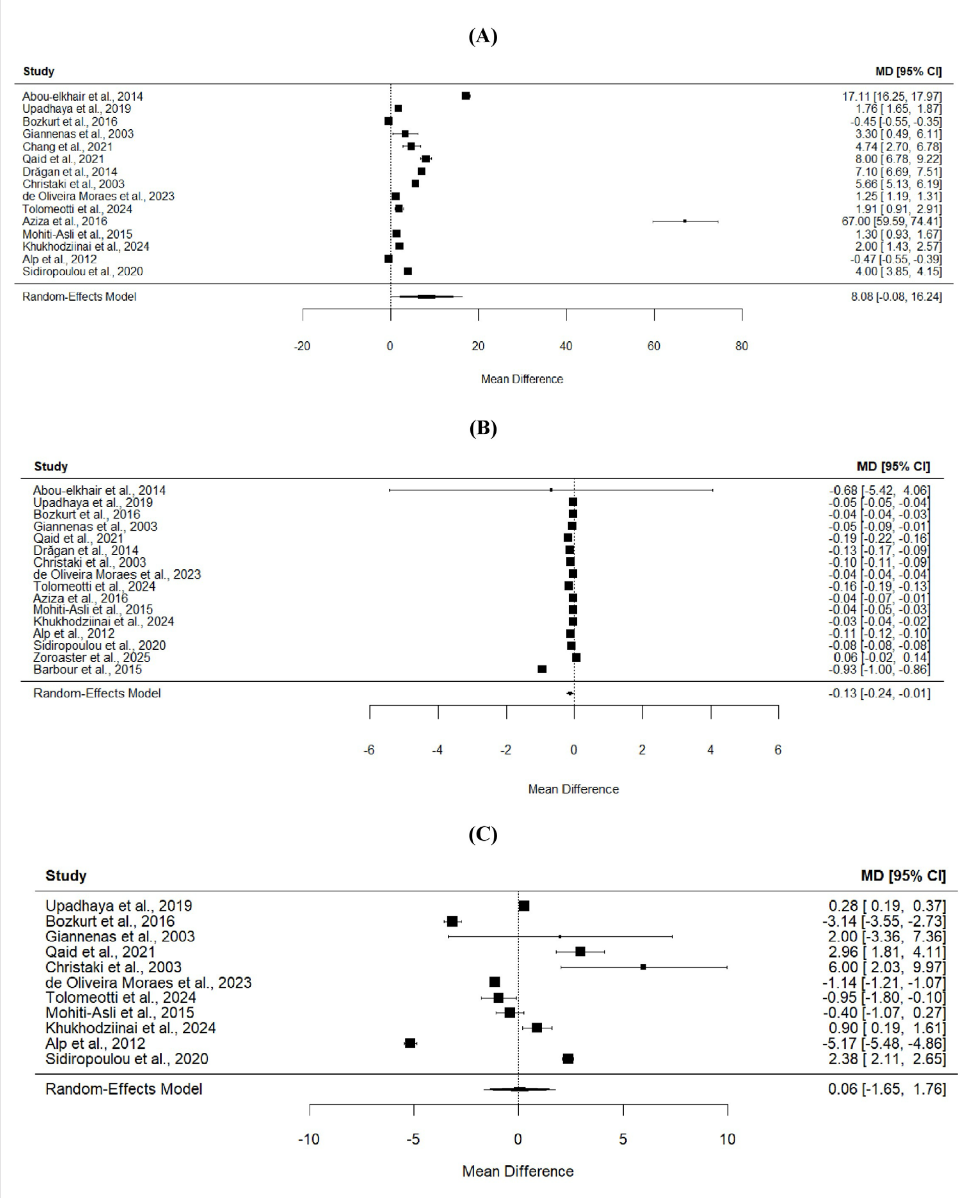

Fig 2. Forest plots for outcomes. Panels: **(A)** Body weight gain (BWG, g), **(B)** Feed conversion ratio (FCR), **(C)** Feed intake (FI). Squares represent study-specific effect estimates with 95% confidence intervals, and square size is proportional to inverse-variance weight. Diamonds represent the pooled random-effects estimate obtained using the restricted maximum-likelihood estimator; horizontal bars extending from the pooled estimate indicate the 95% prediction interval

only blends, or essential oil-containing multicomponent formulations. The latter included formulations in which essential oils were administered with components such as organic acids, benzoic acid, tannins, vitamins, or minerals. These studies were retained as evaluations of practically applied essential oil-based formulations, but their observed effects were not attributed exclusively to the essential oil component. Considerable variation was observed in poultry type, essential oil source, dose, formulation, administration route, supplementation period, and *Eimeria* challenge model.

The essential oil-containing multicomponent formulations were also considered separately in a descriptive assessment. Their findings were not uniform. Some formulations containing essential oils together with benzoic acid, organic acids, or vitamin D3 were associated with improvements in growth, feed efficiency, intestinal health, immune responses, or oocyst reduction. In contrast, the formulation containing essential oils, organic acids, tannins, vitamin E, and zinc showed limited effects under challenge conditions. Because these formulations differed considerably in composition and were represented by few studies within individual outcomes, their results do not permit conclusions regarding the independent contribution of essential oils or any specific accompanying component.

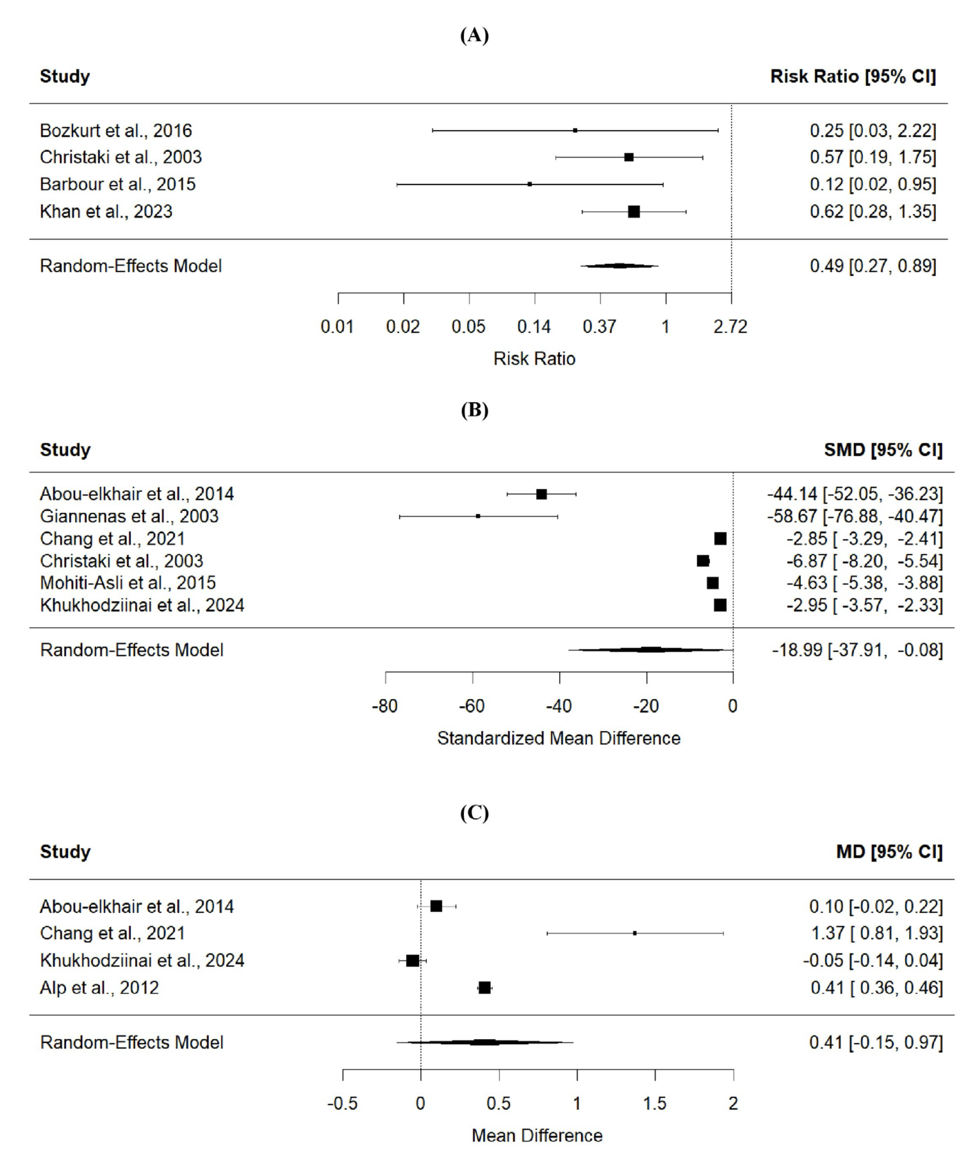

Fig 3. Forest plots for all outcomes. Panels: **(A)** Mortality (%), **(B)** OPG, **(C)** Total protein (g/dL). Squares represent study-specific effect estimates with 95% confidence intervals, and square size is proportional to inverse-variance weight. Diamonds represent the pooled random-effects estimate obtained using the restricted maximum-likelihood estimator; horizontal bars extending from the pooled estimate indicate the 95% prediction interval

Meta-analytical Results

All pooled estimates reported below were obtained using random-effects models with between-study variance estimated by REML. The meta-analytical results obtained for each outcome are summarized in *Table 2*.

Body Weight Gain (BWG)

Fifteen trials (total *N*≈2384 birds) contributed data on BWG. The pooled mean difference was +8.08 g (95% CI −0.08 to 16.24), indicating a trend toward improved body weight gain with essential oil supplementation. In the forest plot *(Fig. 2-A)*, most trials favored higher BWG with essential oils, but effect sizes varied (study-level MDs ranged from slightly negative to strongly positive). Notably, one study [38] reported an unusually large positive effect (≈+67 g), and a few reported small negative differences.

For body weight gain, Egger's regression suggested possible funnel-plot asymmetry (P=0.053), while Begg's test was statistically significant (P=0.0048). These findings may indicate small-study effects; however, they should not be interpreted as definitive evidence of publication bias because the analysis included only 15 studies and the two tests yielded different levels of evidence. The trim-and-fill procedure did not impute any missing studies, and the pooled estimate remained unchanged. Cumulative meta-analysis *(Fig. 5-A)* showed the pooled effect stabilizing around +8 g as studies were added chronologically, with confidence intervals gradually narrowing but still

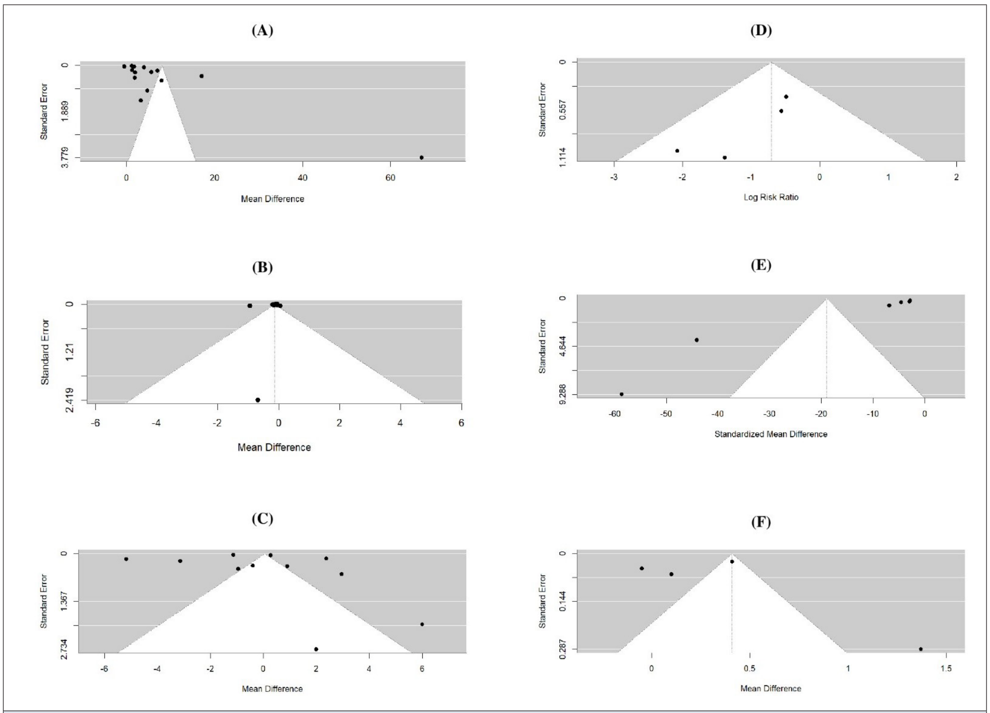

Fig 4. Funnel plots for all outcomes. Panels: **(A)** Body weight gain (BWG, g), **(B)** Feed conversion ratio (FCR), **(C)** Feed intake (FI), **(D)** Mortality (%), **(E)** OPG, **(F)** Total protein (g/dL)

overlapping zero. Influence analysis (leave-one-out) *(Fig. 7)* identified Aziza et al.[38] as an outlier: omitting this trial reduced the pooled estimate by about 4 g. Similarly, Baujat plot *(Fig. 6-A)* inspection indicated that the study by Aziza et al.[38] and a few others [35,36] contributed disproportionately to variability in results.

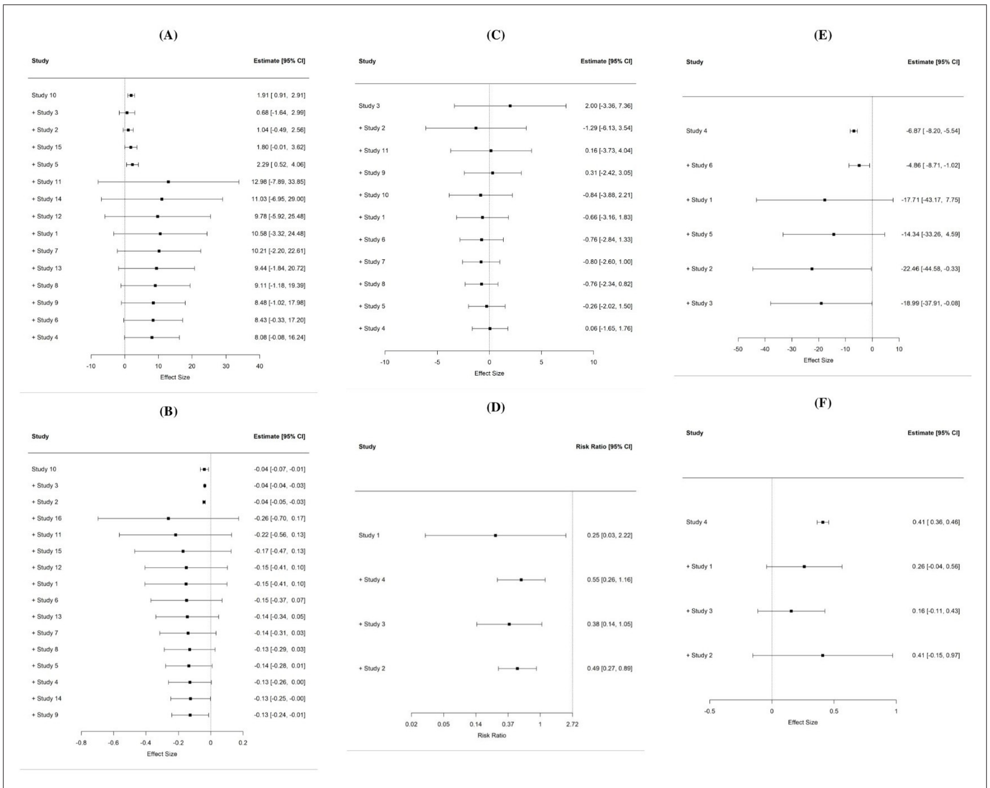

Fig 5. Cumulative meta-analysis across outcomes. Panels: **(A)** Body weight gain (BWG, g), **(B)** Feed conversion ratio (FCR), **(C)** Feed intake (FI), **(D)** Mortality (%), **(E)** OPG, **(F)** Total protein (g/dL)

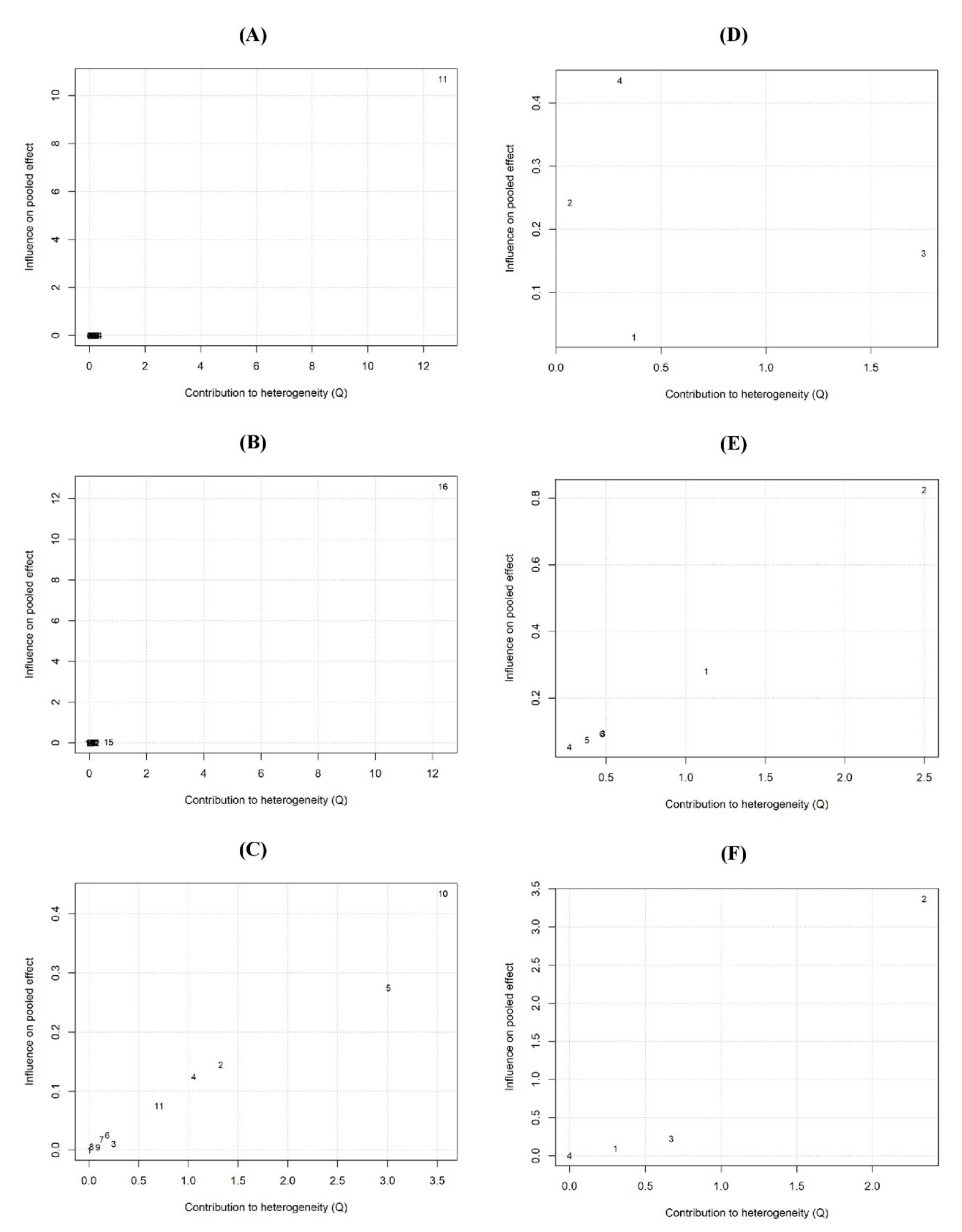

Fig 6. Baujat plots for all outcomes. Panels: **(A)** Body weight gain (BWG, g), **(B)** Feed conversion ratio (FCR), **(C)** Feed intake (FI), **(D)** Mortality (%), **(E)** OPG, **(F)** Total protein (g/dL)

Feed Conversion Ratio (FCR)

Sixteen studies (total $N \approx 2359$) reported FCR. The pooled mean difference was −0.13 (95% CI −0.24 to −0.01), indicating that essential oil supplementation was associated with a modest but statistically significant improvement in FCR (P≈0.032). The forest plot *(Fig. 2-B)* showed that most studies clustered near the null, with several reporting moderate improvements and a few showing slight adverse effects, reflecting variability across trials.

For feed conversion ratio, Egger's regression indicated possible funnel-plot asymmetry (P=0.020), whereas Begg's test was not statistically significant (P=0.079). The disagreement between these tests does not provide conclusive evidence of publication bias and may reflect differences in their statistical properties or the influence of between-study heterogeneity. The trim-and-fill procedure did not impute missing studies, and the adjusted pooled estimate remained unchanged. These findings were therefore interpreted as suggestive of possible small-study effects rather than confirmation of publication bias. Cumulative meta-analysis *(Fig. 5-B)* showed that the direction of effect (favoring essential oils) remained consistent over time, although confidence intervals narrowed only slightly as additional studies were added.

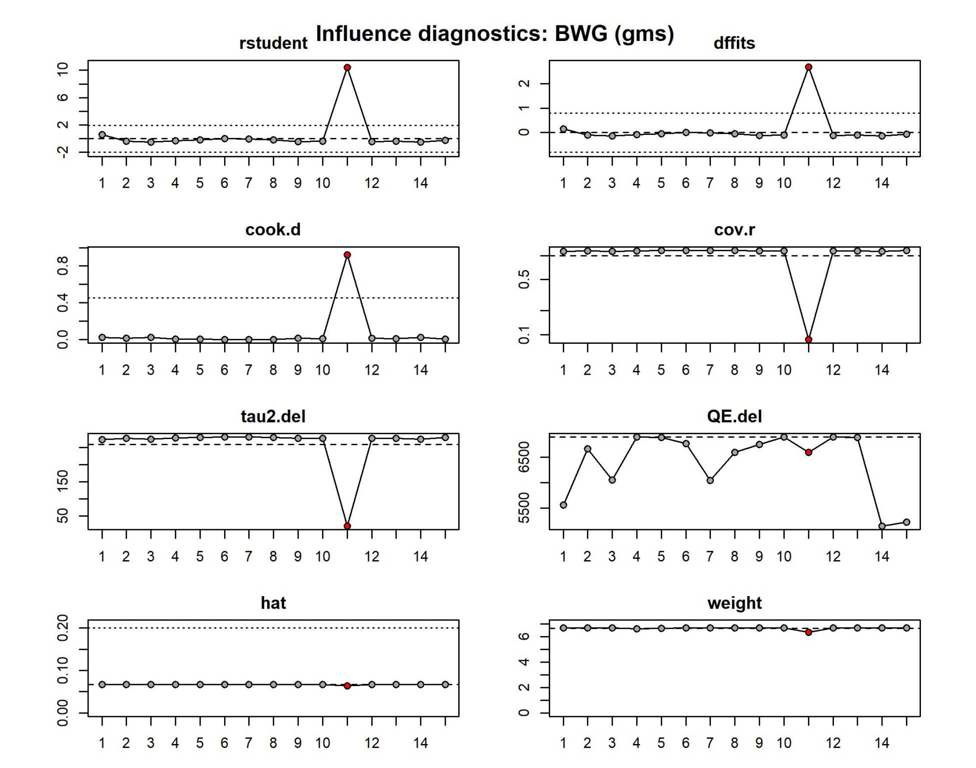

Fig 7. Influence diagnostics for Body weight Gain (BWG, g). Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

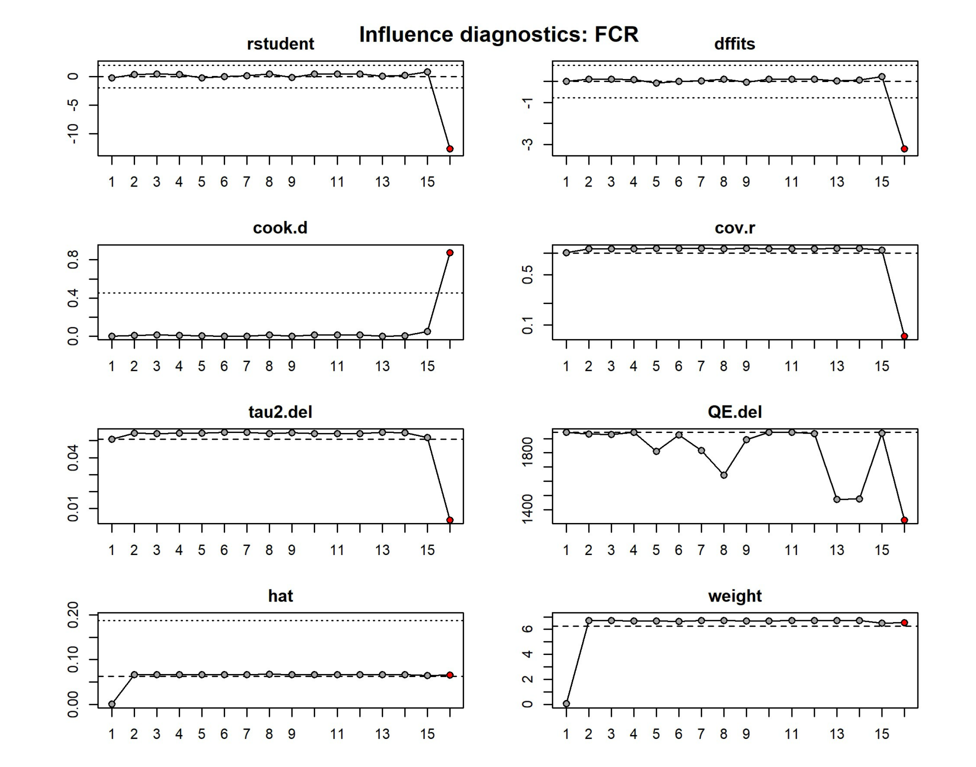

Fig 8. Influence diagnostics for Feed conversion ratio (FCR). Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

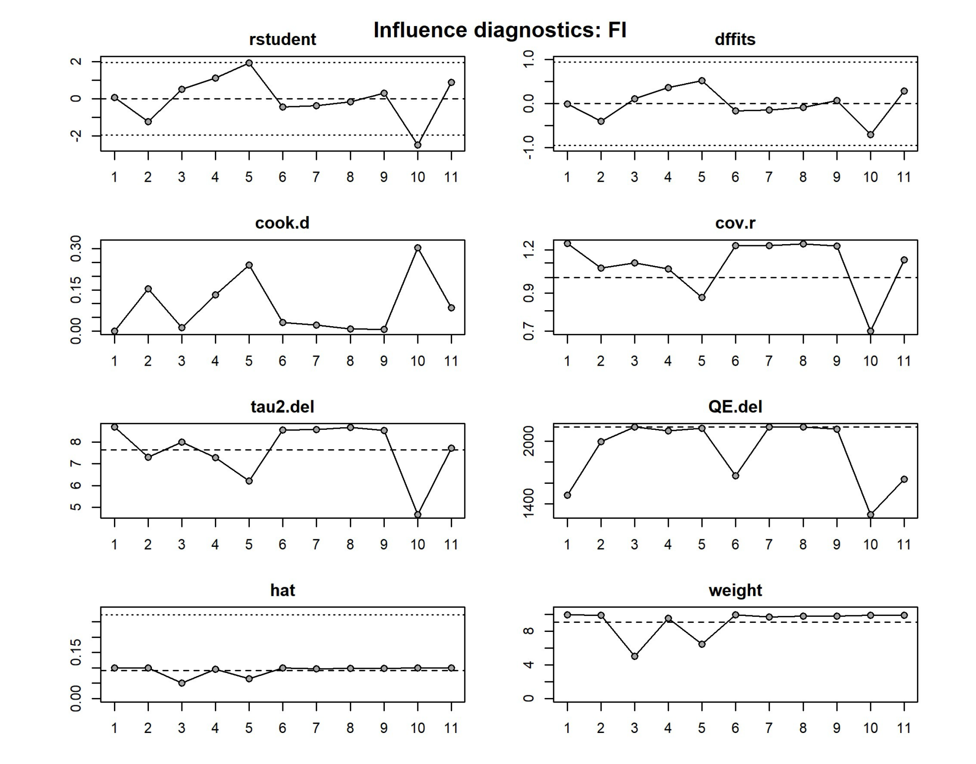

Fig 9. Influence diagnostics for Feed Intake (FI). Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

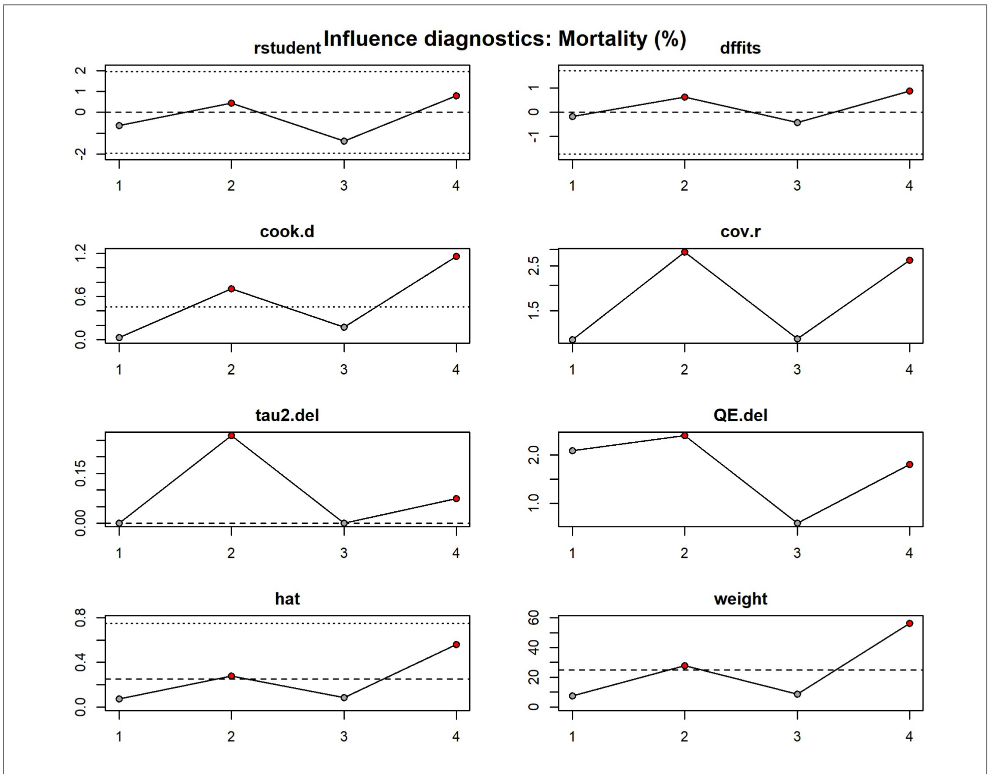

Fig 10. Influence diagnostics for Mortality (%). Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

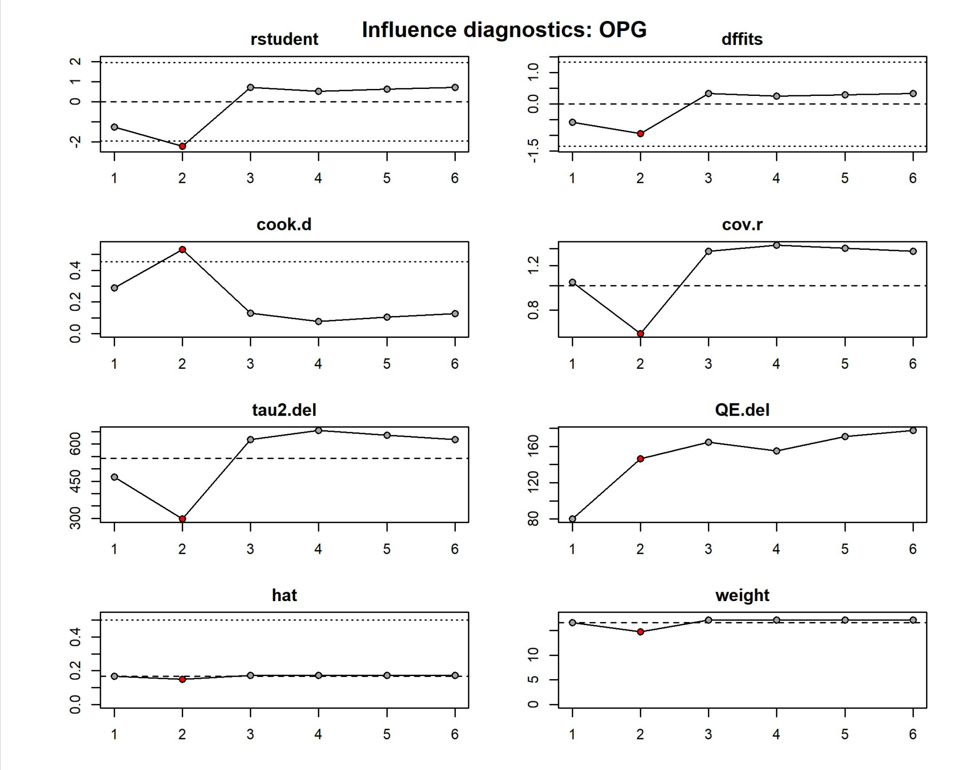

Fig 11. Influence diagnostics for OPG. Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

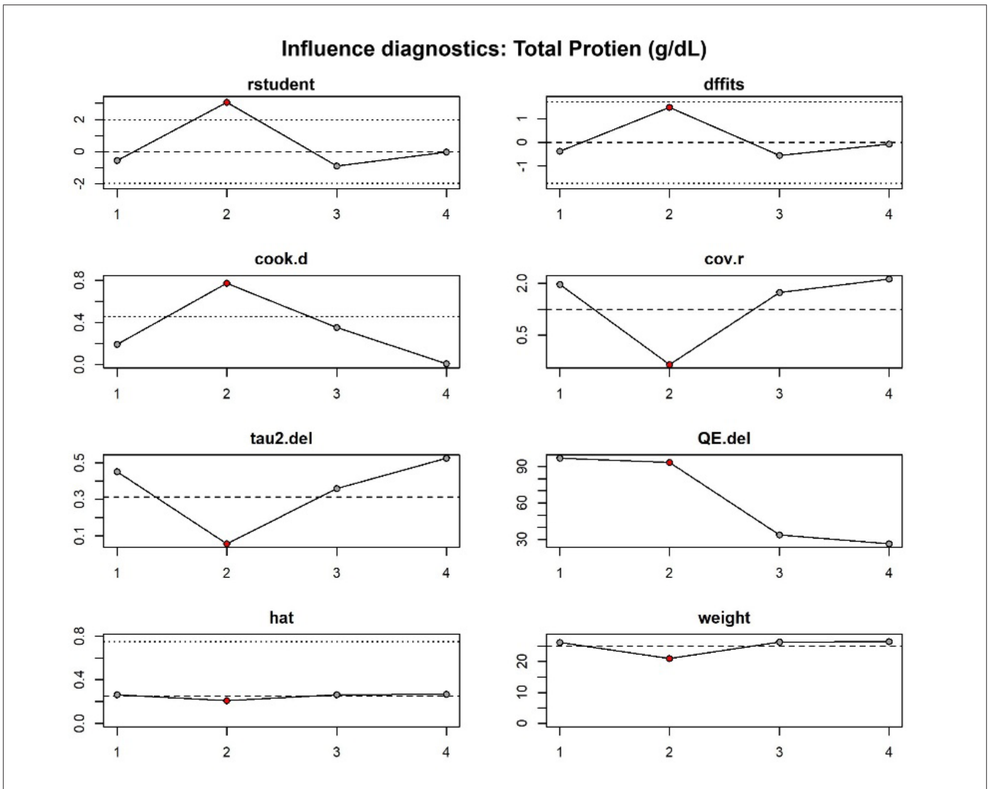

Fig 12. Influence diagnostics for Total Protein (g/dL). Each panel shows the metafor influence diagnostics for the fitted random-effects model, including Cook's distance, hat (leverage), DFFITS, studentized residuals, covariance ratios, and related leave-one-out metrics

Table 1. *Characteristics of the 18 included studies evaluating essential oil-based interventions in poultry, including country, poultry strain, Eimeria challenge, intervention composition and classification, duration, sample size, outcomes, and main findings*

Author (Year)	Country	Poultry Type & Strain	Challenge (Species, Dose)	Intervention Composition, Dose, and Classification	Duration	Outcomes Included in the Meta-analysis	Main Findings
Abou-Elkhair et al. (2014) [23]	Egypt	Broilers	Mixed *Eimeria* spp., 20,000 oocysts	Oregano + Thyme + Garlic EO blend (10-20 mg/kg oregano+thyme, 5-10 mg/kg garlic)	35 d	BWG, FCR, Total Protein, OPG	Improved growth, reduced oocyst shedding, comparable to amprolium
Alp et al. (2012) [24]	Türkiye	Broilers	*Eimeria* spp. (dose not specified)	Oregano essential oil (300 mg/kg feed); individual essential oil intervention	42 d	BWG, FCR, Total Protein, FI	Improved FCR, partial anticoccidial effect, higher IgG
Barbour et al. (2015) [25]	Lebanon	Broilers	8 *Eimeria* spp., 1.76×10^5 oocysts	Eucalyptus + Peppermint oils (0.69 mL/kg BW in water)	Trial period	BWG, FCR, Mortality	Reduced lesions, mortality, oocyst counts, better BWG
Bozkurt et al. (2016) [26]	Türkiye	Broilers (Ross 308)	Mixed *Eimeria* spp., challenge d12	Oregano EO (12–24 mg/kg), Monensin comparison	42 d	BWG, FCR, FI, Mortality	Improved gut health, antioxidant defenses; weaker than monensin
Chang et al. (2021) [27]	China	Laying hens	*E. tenella*, 50.000 oocysts	Garlic EO (0.04–0.08 mL/L in water)	Trial period	BWG, Total Protein, OPG	Reduced lesions, oocyst output, improved IgA/IgM, similar to diclazuril
Christaki et al. (2004) [28]	Greece	Broilers (Cobb-500)	*E. tenella*, 60.000 oocysts	Apacox herbal blend (0.5–1.0 g/kg diet)	35 d	BWG, FCR, OPG, FI, Mortality	Reduced diarrhea, mortality, moderate efficacy vs. lasalocid
de Oliveira Moraes et al. (2023) [29]	Brazil	Broilers (Cobb)	Mixed *Eimeria* spp.	Oregano EO (500 mg/kg), Clove EO (500 mg/kg), combo (250+250 mg/kg)	42 d	BWG, FCR, FI	Improved BWG, FCR, villus/crypt, higher IgA & IgG
Giannenas et al. (2003) [30]	Greece	Broilers	*E. acervulina* & *E. tenella*	Oregano EO (300–600 mg/kg)	Trial period	BWG, FCR, OPG, FI	600 mg/kg improved growth, reduced lesions; similar to salinomycin
Khan et al. (2023) [12]	Pakistan	Japanese quails	*Eimeria tenella*	Oregano EO (200–600 mg/kg)	Trial period	Mortality	Reduced oocysts, lesions, improved growth; weaker than amprolium
Khukhodziinai et al. (2024) [31]	Ukraine	Broilers (Cobb-500)	*E. acervulina, E. maxima, E. tenella, E. necatrix*	Benzoic acid (500 mg/kg), oregano essential oil (500 mg/kg), and their combination; individual EO and EO-containing multicomponent intervention arms	35 d	BWG, FCR, Total Protein, OPG, FI	Combo had best immunity, gut health, reduced oocysts; > salinomycin
Mohiti-Asli et al. (2015) [32]	Iran	Broilers	*E. acervulina, E. maxima, E. tenella* (Livacox overdose)	Oregano EO (300–500 ppm)	Trial period	BWG, FCR, OPG, FI	500 ppm reduced lesions & oocysts, similar to diclazuril
Qaid et al. (2021) [33]	Saudi Arabia	Broilers	Mixed *Eimeria*	EO + organic acid blend	Trial period	FCR, FI	Reduced lesions, improved villi, growth performance
Sidiropoulou et al. (2020) [34]	Greece	Broilers	Mixed *Eimeria*	Oregano and garlic essential oils; essential oil-only blend	Trial period	BWG, FCR, FI	Improved meat quality, antioxidant & immunity
Tolomeotti et al. (2024) [35]	Italy	Broilers	*Eimeria* spp.	EO blend + organic acids + tannins + vitamin E + zinc; EO-containing multicomponent formulation	Trial period	BWG, FCR, FI	Better FCR in starter, breast yield, villi in unchallenged; little effect when challenged
Upadhaya et al. (2019) [36]	South Korea	Broilers	*E. tenella* & *E. maxima* (300–500 oocysts)	EO blend containing eugenol, thymol, and piperine + vitamin D3; EO-containing multicomponent formulation	Trial period	BWG, FCR, FI	Improved BWG, FCR, Lactobacillus counts, lower lesions
Zoroaster et al. (2025) [37]	Italy	Black Livorno chickens	*E. necatrix, E. praecox, E. tenella*	Thymol (5 mg/kg), EO blend EP (50 mg/kg)	Trial period	FCR	*In vitro:* strong inhibition; *In vivo:* reduced OPG after d78, no growth effect
Aziza et al. (2016) [38]	USA	Broilers (Ross 708)	*E. acervulina* & *E. maxima*	*Artemisia annua* leaves (in feed)	Trial period	BWG, FCR	Reduced lesions & oocysts, better growth, immune response
Drăgan et al. (2014) [39]	Romania	Broilers (Ross 308)	*E. tenella*, 10,000 oocysts	*Artemisia annua* leaf powder (1.5% feed), *A. annua* + F. vulgare EO mix (7.5% water)	Trial period	BWG, FCR	Leaf powder: strong reduction in oocysts & lesions; EO mix: moderate effect

***Table 2.** Summary of pooled effect estimates obtained from random-effects models using the restricted maximum-likelihood estimator, heterogeneity tests, prediction intervals, and publication-bias assessments across outcomes*

Outcome	Studies (k)	Egger p	Begg p	Pooled Effect	95% CI (LB-UB)	Q-test p	I^2 (%)	95% Prediction Interval
BWG (gms)	15	0.053	0.0048	8.08	-0.08 - 16.24	<0.001	69.8	-24.51 - 40.66
FCR	16	0.020	0.079	-0.13	-0.24 - -0.01	<0.001	99.0	-0.58 - 0.33
Total Protein (g/dL)	4	0.820	0.497	0.41	-0.15 - 0.97	<0.001	67.0	-0.82 - 1.64
OPG	6	0.0029	0.0048	-18.99	-37.91 - -0.08	<0.001	97.2	-68.44 - 30.45
FI	11	0.938	0.073	0.06	-1.65 - 1.76	<0.001	69.5	-5.63 - 5.74
Mortality (%) (RR)	4	0.092	0.174	0.49	0.27 - 0.89	0.476	0.0	0.27 - 0.89

Influence analysis (leave-one-out) *(Fig. 8)* demonstrated that the pooled result was not driven by any single trial. Baujat plot inspection *(Fig. 6-B)* suggested that a handful of studies contributed disproportionately to the variability in results, particularly those reporting unusually large effects in either direction.

Feed Intake (FI)

Eleven studies (total *N*≈2064) contributed FI data. The pooled mean difference was +0.055 (95% CI −1.655 to 1.765), effectively zero. No statistical significance was observed (CI includes zero). Forest plots *(Fig. 2-C)* showed mixed small effects: studies reported differences ranging from about −5.17 to +6.00 in FI, with most estimates near zero.

Tests for publication bias did not suggest strong asymmetry. Egger's P=0.938 and Begg's P=0.073 indicate no clear bias, though Begg's test was borderline. Trim-and-fill analysis imputed two small studies (K_0=2), which modestly adjusted the pooled MD to −0.723 (95% CI −2.559 to 1.113); this adjustment did not reveal a significant effect. The cumulative meta-analysis *(Fig. 5-C)* showed the pooled estimate remaining near zero as studies accrued, with consistently overlapping CIs. Influence analysis *(Fig. 9)* did not identify any single dominant study (the largest leave-one-out change was <0.5), suggesting no outsized influence. By the Baujat plot *(Fig. 6-C)*, no study stood out dramatically and variability in results appeared to reflect random small differences across trials.

Mortality

Four trials (total N≈672) reported mortality, analyzed as a risk ratio (RR). Essential oil supplementation significantly reduced mortality risk. The pooled RR was 0.493 (95% CI 0.273–0.891), indicating about a 51% relative reduction. This result reached statistical significance, as the 95% CI excludes 1.0. Notably, heterogeneity was negligible (I^2=0%, τ^2=0) and the Q-test P=0.476, suggesting consistent effects across studies. The 95% prediction interval (0.273-0.891) was the same as the confidence interval in this case. The forest plot *(Fig. 3-A)* showed that all studies had RR<1 (favoring essential oils), although two had wide confidence intervals crossing 1.0.

Assessment of funnel-plot asymmetry for mortality was highly uncertain because only four studies contributed data. Egger's test (P=0.092) and Begg's test (P=0.174) were not statistically significant, but the small number of studies provided insufficient power to exclude small-study effects or publication bias. The trim-and-fill procedure imputed one hypothetical study and produced a slightly attenuated pooled risk ratio; however, this adjustment should also be interpreted cautiously because trim-and-fill estimates are unstable when few studies are available. Cumulative meta-analysis *(Fig. 5-D)* showed that early results were variable: adding the final study [28] stabilized the pooled RR around 0.49. Leave-one-out analysis indicated that no single trial was overly influential (the largest change was ΔRR≈−0.14 when omitting Khan et al. [12] *(Fig. 10)*. In the Baujat plot *(Fig. 6-D)*, the modest heterogeneity arose mainly when an outlying study [25] was added, but overall heterogeneity stayed at zero after all studies were included. In summary, essential oils were associated with significantly lower mortality in coccidiosis-challenged birds.

Oocysts Per Gram (OPG)

Six studies (N≈484) provided oocyst count outcomes. The pooled mean difference was −18.99 (95% CI −37.91 to −0.08), indicating significantly lower oocyst shedding with essential oil treatment (negative MD favors essential oil). In the forest plot *(Fig. 3-B)*, all individual study MDs were negative (ranging roughly from −2.85 to −58.67), consistently favoring essential oils, but with one or two studies showing much larger reductions.

For oocyst counts, Egger's regression (P=0.0029) and Begg's test (P=0.0048) indicated funnel-plot asymmetry. These findings raise the possibility of small-study effects or selective publication; however, only six studies were available, limiting the reliability of both tests. Funnel asymmetry may also arise from substantial heterogeneity, differences in outcome measurement, or other methodological variation rather than publication bias alone. The trim-and-fill procedure did not impute missing studies, but this result should not be considered sufficient to exclude reporting bias. Cumulative analysis *(Fig. 5-E)* showed a gradually strengthening negative effect, reaching significance only after the last study [30] was included. Influence analysis *(Fig. 11)* identified Giannenas et al.[30] as highly influential: removing it shifted the pooled MD by +7.04 (toward zero). In sum, the data suggested the potential benefit of essential oils in lowering coccidia shedding.

Total Protein

Four studies (N≈464) reported serum total protein (g/dL). The pooled difference was +0.409 (95% CI –0.155 to 0.972), favoring essential oils but not statistically significant. In the forest plot *(Fig. 3-C)*, one study [27] showed a large positive effect (+1.37), while others were closer to zero.

Egger's test (P=0.820) and Begg's test (P=0.497) did not indicate funnel-plot asymmetry. Nevertheless, only four studies contributed to this outcome, and the absence of statistical significance should not be interpreted as evidence that publication bias was absent. The trim-and-fill procedure did not impute missing studies, and the pooled estimate remained unchanged. Cumulative meta-analysis *(Fig. 5-F)* showed the pooled effect declining from +1.37 (from the first trial) to +0.409 as more studies were added, with confidence intervals tightening but always overlapping zero. Leave-one-out analysis *(Fig. 12)* identified Chang et al.[27] as influential (omitting it increased the pooled estimate by 0.253). Overall, there was no obvious evidence that essential oils significantly altered total protein levels.

General Synthesis

Across the evaluated outcomes, essential oil-based interventions showed variable effects in poultry challenged with *Eimeria* spp. The most consistent finding was a reduction in mortality, while oocyst shedding and feed conversion ratio also showed statistically significant pooled improvements. In contrast, the pooled increase in body weight gain was small and its confidence interval included the null value. Feed intake and serum total protein were not significantly affected. Wide prediction intervals for several outcomes further indicated that the magnitude and direction of effects may differ across intervention formulations and experimental conditions. Overall, the evidence suggests potential benefits for selected coccidiosis-related outcomes but does not support consistent improvement across all measures of growth performance and health.

Discussion

This meta-analysis evaluated the effects of essential oil-based interventions in poultry experimentally challenged with *Eimeria* spp. The pooled findings indicated a modest improvement in feed conversion ratio and reductions in mortality and fecal oocyst shedding. However, the estimated increase in body weight gain was small and statistically uncertain, while feed intake and serum total protein were not significantly affected. These results suggest that essential oil-based interventions may alleviate selected consequences of coccidial infection, particularly mortality, feed inefficiency, and parasite shedding, but they do not demonstrate consistent benefits across all growth and health outcomes.

These findings echo the results of many primary trials. For growth performance, multiple studies have reported that EOs improve weight gain and feed efficiency in coccidiosis-challenged chickens. Mohiti-Asli et al.[32] found that dietary oregano oil *(Origanum vulgare)* significantly increased BW gain and improved FCR in *Eimeria*-infected broilers. Similarly, Lee et al.[40] reported that an encapsulated blend of thymol and carvacrol (major oregano oil constituents) raised BW gain to levels comparable to a non-challenged control in vaccine-challenged broilers. Niknia et al.[41] likewise observed that broilers fed a thymol-carvacrol blend during *Eimeria* challenge had significantly greater BW gain and lower FCR than untreated infected birds. A blend of oregano and garlic oils also produced weight gains and feed efficiency similar to uninfected controls [34]. These improvements in BWG and FCR with EOs are consistent with our meta-pattern: in each case EO-supplemented birds "caught up" to healthy performance. In contrast, sham-challenged positive controls lagged behind. Notably, Chang et al.[27] showed that garlic oil alone prevented the weight loss normally caused by *Eimeria tenella*. They showed garlic EO-treated chickens had body weights significantly higher than untreated infected birds (and similar to those given the anticoccidial drug diclazuril). Taken together, individual trials frequently reported improvements in growth performance, but the pooled evidence was less definitive. Feed conversion ratio improved significantly, whereas the pooled effect on body weight gain remained statistically uncertain because the confidence interval included the null value. Differences in dose, formulation, challenge severity, and study duration may partly explain the variable responses. The findings therefore support a possible performance benefit but do not establish that essential oil-based interventions consistently improve growth under all challenge conditions [32,40,41].

Essential oil treatment also consistently reduced oocyst shedding and gut pathology. In our meta-analysis, EO supplementation was associated with a reduction in fecal oocyst counts. This aligns with reports by Chang et al.[27], who found that garlic EO significantly lowered cecal lesions and oocyst counts in *E. tenella*-infected birds.

Mohiti-Asli et al.[32] similarly showed that broilers fed 500 ppm oregano oil had much lower lesion scores and fecal OPG than infected controls, with values comparable to birds on diclazuril. In study by Sidiropoulou et al.[34], broilers given a premix of oregano + garlic oils excreted significantly fewer oocysts (~0.3-0.5 log unit reductions on days 28 and 37) than untreated birds. Thus, across many trials, EO supplementation reduced parasite load. Mortality was generally low across trials, but pooled results indicated a protective effect of EO treatment.

Beyond these primary outcomes, EOs appear to boost health indices. Coccidiosis typically depresses serum proteins and alters biochemical markers, and there is limited evidence that EOs can counteract this. For example, Chang et al.[27] reported that garlic EO treatment yielded higher levels of IgM, IgG and IgA (immunoglobulins) than controls. It indicated improved immune function and possibly reflecting better serum protein profiles (globulins). However, Lee et al.[40] saw no significant change in total protein with thymol/carvacrol dosing. In our meta, the paucity of serum protein data means we cannot conclusively quantify EO effects on this outcome. Overall, the consensus is that EOs likely mitigate the protein-depleting effect of coccidiosis by improving gut integrity and reducing inflammation.

The ability of EOs to alleviate coccidial effects is biologically plausible given their well-established antimicrobial, anti-inflammatory, and immunomodulatory properties. Many EOs contain phenolic compounds (e.g. thymol, carvacrol, eugenol, cinnamaldehyde, allicin) that are known to be lethal to pathogens. *In vitro* experiments confirm that these compounds directly damage *Eimeria* parasites: for instance, thymol and carvacrol at ≥2% concentration destroyed 90-96% of *E. tenella* oocysts and inhibited sporulation [5]. Piper betle leaf oil (rich in eugenol and terpenes) similarly caused dose-dependent inhibition of oocyst sporulation and wall disintegration *in vitro. In vivo,* such actions would translate to fewer infective oocysts and reduced cecal invasion. These antimicrobial effects are complemented by the broader activity of EOs against gut pathogens and microbiota: many studies (including the BA+OEO trial) noted that EOs suppressed harmful bacteria *(Salmonella, E. coli, Clostridium)* while boosting beneficial Lactobacilli. This shift in the microbiome can enhance gut health and indirectly reduce the severity of coccidiosis [31].

EOs also exert strong immunomodulatory and anti-inflammatory effects that support resistance to *Eimeria*. Several studies highlight that EO components stimulate host immunity: for example, cinnamaldehyde supplementation increased intestinal cytokine gene expression (IL-1β, IL-6, IL-15, IFN-γ) and reduced weight loss and oocyst shedding in infected birds [42]. In the BA+OEO study, oregano oil upregulated interferon-γ (IFN-γ) mRNA and suppressed interleukin-10 and TLR-4 expression, while enhancing *Eimeria*-specific IgY antibody titer [31]. These immunostimulatory effects can fortify the host's defenses, helping control the parasite. At the same time, EOs' anti-inflammatory action protects intestinal tissues: by quenching oxidative stress and moderating pro-inflammatory signaling, EOs reduce tissue damage that would otherwise impair digestion and absorption [43] .

The results of our meta-analysis carry significant implications for commercial poultry production, particularly under conditions where the use of conventional drugs is restricted (e.g., organic or antibiotic-free systems). Resistance to traditional coccidiostats (ionophores and chemicals) is increasing, while consumer demand for antibiotic-free poultry products continues to rise [44]. Essential oil-based interventions may have potential as complementary components of anticoccidial-control programs, although the present evidence is insufficient to establish them as direct replacements for conventional anticoccidial agents. The BA+OEO trial explicitly concluded that oregano oil plus benzoic acid "can substitute for a commercial anti-coccidial agent (salinomycin)" by controlling coccidiosis and enhancing growth and immunity. Similarly, the encapsulated thymol/carvacrol blend was as effective as salinomycin in a vaccine-challenge model [40]. Thus, selected essential oil-based formulations may be considered for integration into feed as part of broader coccidiosis-management strategies, but their effectiveness should be confirmed through standardized comparative and field-based trials. They may also pair well with vaccines; for example, encapsulated oils offset the temporary growth depression often seen after coccidiosis vaccination. Moreover, because EOs can improve general gut health and microbiota balance, they could provide broad performance benefits in intensive systems [41,42].

However, practical adoption requires addressing challenges. The cost and consistency of EOs vary widely; high-quality, food-grade oils can be expensive. Regulatory approval for the use of plant extracts in organic systems is another hurdle. Also, effects may differ by species and environment [7]. Nevertheless, the literature suggests that EOs can fit into antibiotic- and anticoccidial-free programs: for example, synbiotic blends of probiotics and EO extracts have been shown to ameliorate coccidiosis impacts in broilers, improving intestinal health [42]. In summary, EOs represent a promising tool for sustainable poultry production, but they should be viewed as part of an integrated management strategy (along with vaccination, biosecurity, nutrition) rather than a standalone cure.

The main challenge in interpreting these findings is the variability among studies. Differences in EO source, dose, formulation, delivery method, bird strain, age, housing, and infection model contributed to between-study heterogeneity. Some trials used mild vaccine challenges, while others employed high-dose *Eimeria* inoculations of mixed species, leading to differences in disease severity and response to EOs. Moreover, many trials were small (few pens) and measured outcomes only over a short window (often 1-2 weeks post-infection). Another limitation is the diversity in reported outcomes. Although subgroup analysis and meta-regression could potentially identify sources of heterogeneity, the limited number of studies per outcome and the inconsistent reporting of essential oil composition, dose, administration route, and challenge characteristics precluded meaningful moderator analyses. While most trials assessed growth and oocyst counts, relatively few included immunological, microbiological, or detailed histological parameters, restricting deeper understanding of mechanisms.

Possible publication bias should also be considered when interpreting the pooled findings. Funnel-plot asymmetry and statistical tests suggested potential small-study effects for body weight gain and, more clearly, for oocyst counts. Therefore, the pooled estimates for these outcomes may partly overestimate the true effects of essential oils, particularly if small studies reporting null or unfavorable results remain unpublished. Nevertheless, publication-bias tests have limited reliability when few studies are available, and the trim-and-fill analysis did not identify missing studies for either outcome. Accordingly, the observed effects, especially the reduction in oocyst shedding, should be interpreted cautiously rather than regarded as definitive. Some studies did find minimal effects of EOs on growth - for example, Zoroaster et al.[37] reported no significant improvement in ADG or FCR with low-dose thymol/carvacrol *in vivo*, despite potent *in vitro* effects. A specific limitation is the lack of standardization in challenge protocols. Some used live commercial vaccines (attenuated *Eimeria),* others used purified sporulated oocysts of one or more species. The intensity of infection and timing relative to measurements varied. For instance, Mohiti-Asli & Ghanaatparast-Rashti [32] challenged with a vaccine and measured on day 28, whereas Chang et al.[27] infected with *E. tenella* oocysts and measured over 7 days. These differences influence the apparent efficacy of EOs. A formal risk-of-bias assessment of the included studies was not performed, which represents an important limitation of this meta-analysis. Several primary studies provided insufficient information regarding random sequence generation, allocation concealment, blinding of investigators or outcome assessors, and selective outcome reporting. Consequently, the methodological quality and internal validity of the included evidence could not be systematically quantified, and the pooled estimates should therefore be interpreted with appropriate caution.

Future research should prioritize adequately powered, standardized, and long-term trials. Experimental protocols should clearly define poultry species, strain, age, housing conditions, *Eimeria* species, inoculation or vaccine dose, challenge timing, and outcome-assessment periods. Essential oil source, chemical composition, formulation, dose, and administration route should also be reported consistently. Trials should evaluate essential oil-only interventions separately from multicomponent formulations to allow the independent effects of essential oils to be determined. In addition to growth performance and oocyst shedding, future studies should assess lesion scores, intestinal histology, microbiota composition, inflammatory and immune markers, product quality, safety, and economic feasibility. Field-based studies conducted under commercial production conditions are particularly needed to determine whether the observed experimental effects translate into practical benefits.

Conclusion

This meta-analysis indicates that essential oil-based interventions were associated with a modest improvement in feed conversion ratio and reductions in mortality and oocyst shedding in poultry challenged with *Eimeria* spp. The pooled effect on body weight gain was statistically uncertain, while feed intake and serum total protein were not significantly affected. The findings therefore do not demonstrate consistent benefits across all evaluated outcomes. In addition, because some interventions contained other active components, their effects cannot be attributed exclusively to essential oils. Essential oil-based interventions may have potential as components of integrated coccidiosis-management programs, but further standardized, adequately powered, and field-based studies are required before firm conclusions regarding their practical efficacy can be made.

Declaration

Availability of Data and Materials: All data analyzed in this study were obtained from the published articles cited in the manuscript. The extracted dataset used in the meta-analysis is available from the corresponding author upon reasonable request.

Acknowledgements: The author has no acknowledgements to declare.

Competing Interests: The author declares that there are no commercial, financial, or personal relationships that could be construed as potential competing interests.

Declaration of Generative Artificial Intelligence: No GenAI was used in preparation of the manuscript.

Authors' Contributions: Ayed Alshammari: Conceptualization, data curation, formal analysis, project administration, supervision, writing-original draft, and writing-review and editing. The author read and approved the final version of the manuscript.

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