

A Meta-Analysis of Plant-Derived Essential Oil Supplementation in Pre-weaned Holstein Calves: Growth Performance, Fecal Score, Feed Efficiency, Immunity and Ruminal Parameters

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ABSTRACT

Antibiotics have long been used in livestock production for disease prevention, treatment, and growth promotion; however, their use has been increasingly restricted in many countries due to concerns regarding antimicrobial resistance and food safety. Consequently, interest has grown in natural feed additives such as essential oils (EOs), which are known for their antimicrobial, antioxidant, and immunomodulatory properties. The present study aimed to systematically evaluate the effects of EO supplementation on immunity, growth performance, fecal score, feed efficiency, and rumen fermentation parameters in pre-weaned dairy calves by conducting a comprehensive meta-analysis of available research findings. The results demonstrated that EO supplementation significantly increased average daily gain (ADG) ($P < 0.05$) in pre-weaned Holstein calves. Although feed efficiency and fecal scores were improved in EO-treated calves, neither difference reached statistical significance ($P = 0.089$ and $P = 0.093$, respectively). Importantly, EO administration markedly increased serum IgA ($P < 0.05$) and IgG concentrations ($P < 0.001$), indicating enhanced immune function. Body measurements remained unaffected by EO supplementation. Rumen fermentation parameters, including total volatile fatty acids, pH, acetate, butyrate, and propionate, showed no significant differences; however, ruminal ammonia-N concentration decreased significantly with EO use ($P < 0.05$). Overall, the findings suggest that EOs play a beneficial role in improving growth performance, enhancing protein utilization, reducing nitrogen waste, and strengthening immune responses in pre-weaned dairy calves, providing a promising natural alternative to antibiotics for early-life calf management.

Keywords: Essential oil, Immunity, Holstein calves, Meta-analysis, Rumen parameters.

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1. Introduction

Antibiotics have been used extensively in farmed animals for decades to treat various diseases and to help promote growth. However, their widespread use in agriculture and livestock has raised concerns about antibiotic residues in food, contributing to the natural evolution of antibiotic resistance in pathogens (Van *et al.*, 2020; Okaiyeto *et al.*, 2024). Antimicrobial resistance has been identified as one of the top three threats to public health by the World Health Organization (WHO) (Salam *et al.*, 2023). This has led many countries to restrict antibiotics used in food-producing animals (Ma *et al.*, 2021). As a result, scientists and producers have been searching for alternative natural feed additives that can be replace antibiotics to avoid the economic losses that would be caused by these restrictions. Essential oils from plants, probiotics, prebiotics and acidifiers are the most important alternative feed additives in the livestock diet (Luo *et al.*, 2024). Essential oils are aromatic extracts obtained from a diverse range of plants. Depending on the type of plant from which they are extracted, the composition of these oils varies. It is estimated that they contain more than 200 bioactive compounds (Pezantes-Orellana *et al.*, 2024). Due to their high content of volatile, aromatic and bioactive compounds (terpenoids and terpenes), EOs and extracts have gained considerable popularity in various industries in recent years (Bolouri *et al.*, 2022). These compounds are also important in the pharmaceutical, food, agriculture, personal care and healthcare industries for their inherent antioxidant and antimicrobial properties (Aćimović *et al.*, 2022). In livestock enterprises, the health and well-being of the animals in the herd is an important factor in the profitability of the enterprises. Diseases cause high yield losses on livestock farms. They are one of the main sources of stress for animals (Özdemir *et al.*, 2022). Calves are highly vulnerable to diseases, primarily because their immune system is not fully developed in the first weeks of their lives. Diarrhea is the primary cause of death in dairy calves and significantly contributes to morbidity and mortality during the early stages of their lives (Schild *et al.*, 2020; Carter *et al.* 2021; Hordofa *et al.*, 2021; Abebe *et al.*, 2023). Calves that survive diarrhea often face several other problems, including reduced weight gain and increased susceptibility to other diseases (Lora *et al.*, 2018; Schinwald *et al.*, 2022; Özdemir and Yanar 2024). Diarrheal calves, regardless of the cause of diarrhea, often have an overgrowth of pathogenic microorganisms in the small intestine. Oral administration of antimicrobials to diarrheal calves may have a beneficial effect on disease outcome by reducing the duration and severity of disease (Katsoulos *et al.*, 2017). The EOs extracted from certain plants can have powerful antimicrobial properties and can help maintain gut health and prevent diarrhea in calves (Liu *et al.*, 2020), thereby increasing animal growth and performance. The effectiveness of different EO sources in improving average daily

gain (ADG) (Liu *et al.*, 2020; Soltan 2009; Ebrahimi *et al.*, 2017; Tapki *et al.*, 2020; Biyik *et al.*, 2023; Kara and Pirici 2024) and feed efficiency (Tapki *et al.*, 2020; Nora *et al.*, 2024) has been reported. In addition, EOs have been reported to be effective in improving the immune status of calves to fight potential diseases and pathogens by increasing immunoglobulin levels (Liu *et al.*, 2020; Biyik *et al.* 2023; Swedzinski *et al.*, 2020). Rumen development is highly critical for young calves to the transition to solid feed, and volatile fatty acids (VFAs) produced by microbial fermentation are essential energy sources and stimulants for this process. Acetate (Biyik *et al.*, 2023), butyrate (Vakili *et al.*, 2013; Akbarian-Tefaghi *et al.*, 2018), propionate as well as total VFAs (Biyik *et al.*, 2023) have also been reported to increase with EO administration in dairy calves. However, there are also conflicting results in the literature for all these parameters, where EO administration was found to be ineffective. Considering the conflicting results from various studies on this topic, we propose that a meta-analysis could offer a comprehensive understanding of the overall effects of EO administration on dairy calves. This analysis would provide valuable insights to optimize calf raising strategies during the critical pre-weaning period. In this meta-analysis it was aimed to comprehensively evaluate the effects of EO supplementation on immunity, growth, fecal score, feed efficiency and rumen parameters of pre-weaning Holstein calves through a systematic synthesis of available research data.

2. Materials and Methods

2.1 Literature Review and Selection of the Studies

PRISMA guidelines were utilized for the identification, selection and inclusion of the references to ensure an accurate meta-analysis (Moher *et al.*, 2009). The strategy used to search for information is shown in Figure 1. A systematic search of PubMed, Science Direct, Web of Science and Scopus databases identified studies evaluating the effects of EO supplementation on immunity, growth, development, feed efficiency, fecal score and rumen parameters in Holstein calves before weaning. The keywords ‘calves’, ‘essential oil’, ‘performance’, ‘rumen fermentation’, ‘dairy calves’, ‘pre-weaning’, ‘immunity’ were used in article selection. No language discrimination was made in the articles used in the study. In addition to the full text articles published in English, one article was published in Persian (Ebrahimi *et al.*, 2017), but the tables and the abstract were provided in English, therefore the results of the study were also included in the analysis. To evaluate publications to be included in the study, titles and abstracts were reviewed. Peer-reviewed journal articles meeting the eligibility criteria were included. Conference proceedings, conference abstracts, and other non-peer-reviewed publications were

excluded from the evaluation. For comparability, only studies that met the following selection criteria were included. 1) Only studies on the period before weaning were included. 2) Only studies conducted in Holstein breeds were included. 3) Studies were only included if they investigated the effect of EO on the variables of interest, regardless of type. 4) The selected studies reported the least squared means of the control and experimental treatment data with at least one measure of variance (standard errors or standard deviations).

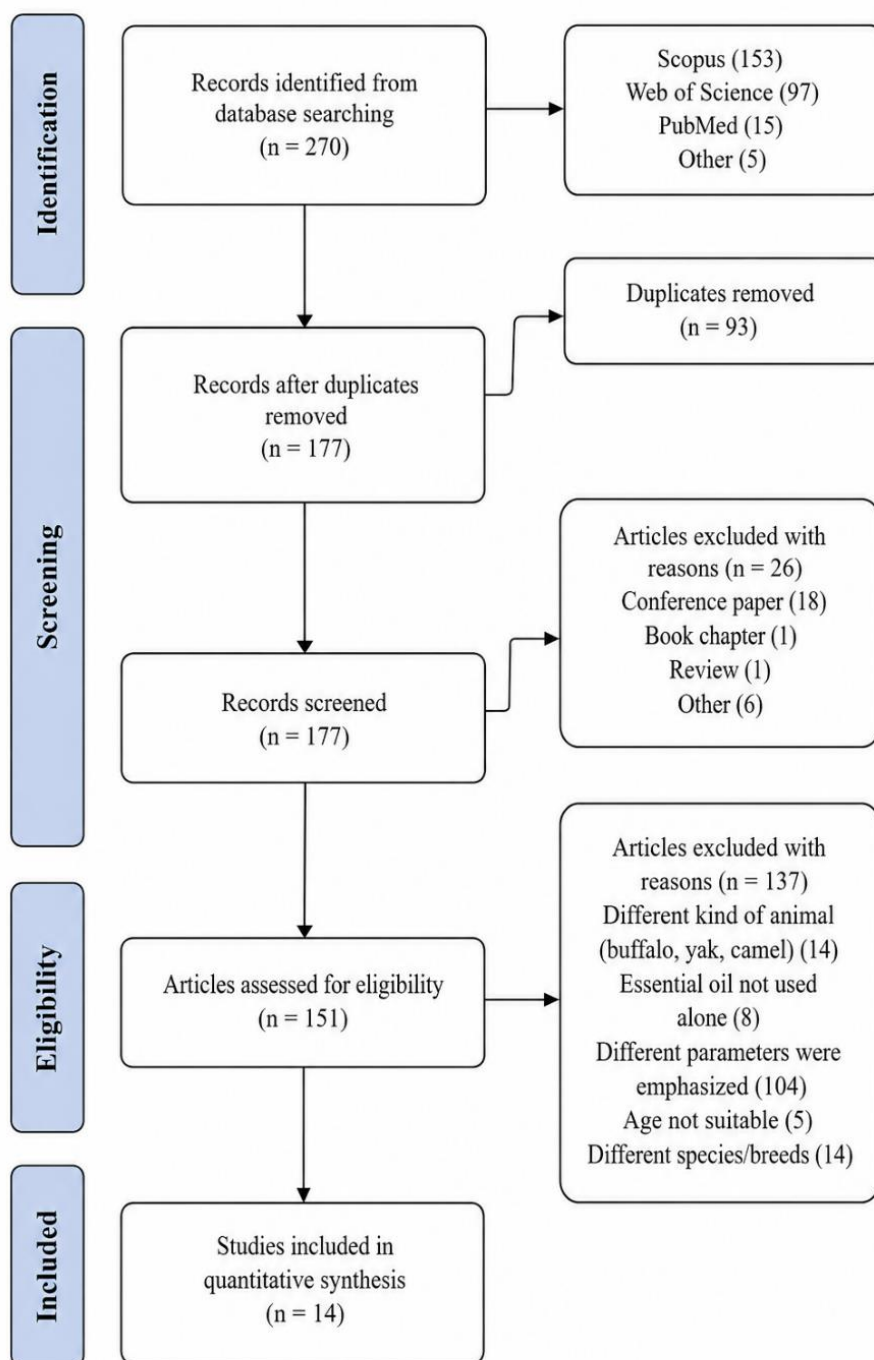


Figure 1. Flow chart of the literature search and article selection procedure.

2.2 Data Extraction

In the first stage of the screening process, 270 records were identified. Among these records, 93 were removed as duplicates. Of the remaining 177 records, 26 were excluded because they were published as book chapters, conference proceedings, or other non-eligible publication types; 5 were excluded because the study materials did not correspond to the pre-weaning period or the weaning age was substantially different from that of the other studies; 104 were excluded because they did not report parameters eligible for the present meta-analysis; and 14 were excluded because different species or breeds were used as experimental materials. Consequently, 14 articles met all inclusion criteria and were included in the final evaluation. (Figure 1). For inclusion in the meta-analysis, variables needed to be reported in a minimum of three distinct studies (Orzuna-Orzuna *et al.*, 2021a; Orzuna-Orzuna *et al.*, 2021b; Belanche *et al.*, 2020). Studies investigating plant-derived essential oils, either administered as a single essential oil or as part of a blend, were considered eligible. No distinction was made based on the method of administration. Some researchers examined more than one essential oil source in a single study (Akbarian-Tefaghi *et al.*, 2018; Ebrahimi *et al.* 2017; Vakili *et al.*, 2013) or used more than one dose (Asghari *et al.*, 2021; Biyik *et al.*, 2023; Jahani-Azizabadi *et al.*, 2022; Kara and Pirci 2024; Luo *et al.*, 2024; Özkaya *et al.*, 2023; Soltan 2009). The eligible interventions included single essential oils as well as blends containing more than one plant-derived essential oil. The form or method of administration was not used as a criterion for distinguishing eligible interventions. A total of 14 studies met the predefined eligibility criteria and were included in the meta-analysis. Because several studies evaluated multiple essential oil sources and/or dose levels, individual studies could contribute more than one treatment–control comparison to a given outcome. The numbers of treatment–control comparisons were 26 for ADG, 12 for feed efficiency, 10 for fecal score, 11 for IgG, 3 for IgA, 5 for IgM, 12 for withers height, 9 for heart girth, 5 for body length, 4 for hip width, 8 for rumen pH, 5 for total VFA, 7 for acetate, 8 for butyrate, 7 for propionate, and 9 for ruminal NH₃. In the present study, growth and development parameters (ADG, feed efficiency and fecal score), immunoglobulins (IgM, IgG, IgA), body measurements (withers height, heart girth, body length, hip width), and rumen parameters (rumen pH, total VFA, acetate, butyrate, propionate, NH₃) were analyzed. In the selected studies calves were supplemented with EO in specified quantities daily throughout the pre-weaning period. In the analysis of immunoglobulin levels, rumen parameters and body measurements and the fecal score the values reported at weaning was considered in the analysis. While the pre-weaning period averages were analyzed for the ADG, feed efficiency. The articles using the formula total BW gain (kg) / dry matter intake (DMI) (kg) for the calculation

of feed efficiency were selected while organizing the data. The publications included in the study, the number of animals used in these studies, EO sources added to the diet, EO doses and the outcomes of these studies utilized in the meta-analysis are presented in Table 1.

Among the selected studies, there were articles that used different units of measurement in the treatment groups tested. Therefore, the units of the treatments in the same group were standardized. In some of the studies used, SEM (standard error of treatment means) value was given instead of standard deviation (SD) value. These data were converted to SD and standardized by utilizing the below formula.

$$SD = SEM \times \sqrt{n} \quad (1)$$

$$SE = SD \times \sqrt{n} \quad (2)$$

SEM = standard error of treatment means, n = number of replicates.

SE= standard error.

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Table 1. Information from the included studies on the effects of essential oils in pre-weaning Holstein calves.

Studies	Country	N	Weaning Age	Treatment	Dose	Variables	Route of administration
Akbarian-Tefaghi <i>et al.</i> , (2018)	Iran	22	55	<i>Thyme oil</i> <i>Eucalyptus oil</i> <i>Celery oil</i>	23 g/kg DM	ADG, FS, WH, HG, ph, NH ₃ , B	Starter feed
Asghari <i>et al.</i> , (2021)	Iran	40	42	Blend (<i>Thymus kotschyanus</i> , <i>Lavandula angustifolia</i> , <i>Salvia officinalis</i> and <i>Capparis spinosa</i>)	100 mg, 200 mg, 300 mg/day	ADG, FE	Milk
Biyik <i>et al.</i> , (2023)	Türkiye	40	56	<i>Rosemary oil</i>	500 mg, 1000 mg, 2000 mg/day	ADG, IgG, ph, NH ₃ , A, P, VFA	Milk replacer
Coelho <i>et al.</i> , (2023)	Brasil	40	56	Blend (<i>Peppermint</i> , <i>eucalyptus</i> , and <i>menthol crystals</i>)	4 mL/day	ADG, FS, WH, HG, HW	Milk replacer
Ebrahimi <i>et al.</i> , (2017)	Iran	24	60-65	<i>Mint oil</i> <i>Thyme oil</i>	% 0.2/kg BW	ADG, FS	Starter feed
Jahani-Azizabadi <i>et al.</i> , (2022)	Iran	48	58-59	Blend (<i>Echinacea angustifolia</i> , <i>Achillea millefolium</i> , and <i>Mentha piperita</i>)	4 mL, 8 mL, 12 mL/day	ADG, IgG, WH, HG, BL, NH ₃ , B	Milk
Kara and Pirci (2024)	Türkiye	24	70	<i>Salvia officinalis</i>	100 µL, 200 µL/day	ADG, FE, FS, IgM, IgG, WH, HG, HW, BL	Milk
Luo <i>et al.</i> , (2024)	China	24	56	<i>Oregano oil</i>	4 mL, 6 mL, 8 mL/d	IgA, IgM, IgG	Oral gavage
Özkaya <i>et al.</i> , (2023)	Türkiye	48	NA	<i>Oreganum onites</i> EO byproduct / Hydrosol	40 ml, 60 ml, 80 ml/d	ADG, FE, WH	Milk
Ritt <i>et al.</i> , (2023)	Brasil	10	53	<i>Oregano oil</i>	60mg/kg BW/day	ADG, FE, A, P, VFA	Milk replacer
Selvi and Tapki (2019)	Türkiye	44	67-68	<i>Oregano oil</i>	893 mg/day	ADG, FE, FS	Milk replacer
Soltan (2009)	Egypt	100	56	Blend (<i>Eucalyptus</i> , <i>menthol crystal</i> , <i>mint</i>)	94 mg, 187 mg, 281 mg/day	ADG	Milk replacer
Tapki <i>et al.</i> , (2020)	Türkiye	28	55-60	<i>Oregano oil</i>	150mg/l	ADG, FE, FS	Milk
Vakili <i>et al.</i> , (2013)	Iran	8	45	<i>Thyme oil</i> <i>Cinnamon oil</i>	5g/day	pH, NH, B	Diet

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A, Acetate; ADG, Average Daily Gain; B, Butyrate; BL, Body Length; BW, Body Weight; FS, Fecal Score; FE, Feed Efficiency; HG, Heart girth; HW, Hip width; MR, Milk Replacer; P, Propionate; VFA, Volatile Fatty Acids; WH, Withers Height, Ig; Immunoglobulin, **N; number of animals.**

2.3 Study Quality Assessment

Two reviewers independently evaluated the risk of bias for each study using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool for animal intervention studies (Hooijmans et al., 2014). The assessment covered 10 domains across selection, performance, detection, attrition, reporting, and other biases. Each domain was classified as having a low, high, or unclear risk of bias according to SYRCLE guidelines. Disagreements were resolved through consensus or by consulting a third reviewer. The detailed domain-by-domain risk of bias assessment for all included studies is provided in Supplementary Table S1. Overall, all included studies demonstrated a low risk of attrition, reporting, and detection biases, alongside homogenous baseline characteristics, although performance and selection biases were predominantly judged as unclear due to limited reporting of blinding and randomization procedures in the original texts.

2.4 Statistical Analysis

In meta-analyses, a random or fixed model was used to examine differences between means. The selection of the model was based on the proportions, contingent upon whether the results were homogeneous or heterogeneous (Hintze and Kaysville 2007). The fixed model was used for homogeneous outcomes and the random model for heterogeneity. Heterogeneity was estimated using the I^2 statistic ($\alpha=.10$ for heterogeneity analysis). In order to assess the difference between the treatments comparisons analyzed in the control group, standard mean differences (SMDs) and standard errors with 95% confidence intervals (CI) were calculated. For SMD results, Cohen's method was preferred if there were many studies ($n > 10$) and Hedges' method if there were fewer than 10 studies. In the case of SMDs, the Cohen's method was used to assess the effect size. Publication bias was assessed using Begg's test to determine the presence of potential publication bias among the studies investigating the effects of EO supplementation on the evaluated traits. Funnel plots were also used for the visual assessment of publication bias (Duval and Tweedie, 2000). STATA statistical software (version 11.2; Stata Statistical Software, Stata Corp LLC, Texas, USA) was used for all statistical analyses.

3. Results

The pooled effect sizes of the various parameters examined in the meta-analytic review of studies evaluating the effects of EO administration on development, growth, immunity and fecal score of calves is shown in Table 2. It shows the SMD, the 95% CIs and the statistics of heterogeneity (I^2) for each of the outcomes. Findings highlight both overall efficacy of EO for

calf performance and interstudy variation using heterogeneity measures. Substantial heterogeneity was found for several outcomes, indicating variability in the magnitude of effects between studies, likely due to differences in experimental design, animal characteristics and environmental conditions.

Table 2. Pooled effect sizes of essential oil administration on growth, development, feed efficiency, immunity, rumen parameters of calves.

Variables	N	Effect Size			Heterogeneity	
		SMD	95% CI	P	I ²	P
Average Daily Gain	26	0.40	[0.14, 0.66]	0.003	59.3%	< 0.001
Feed Efficiency	12	0.29	[-0.04, 0.49]	0.089	7.3%	0.375
Fecal Score	10	-0.34	[-0.73, 0.06]	0.093	55.5%	0.017
Immunoglobulin G	11	0.46	[0.18, 0.75]	0.001	0.0%	0.925
Immunoglobulin A	3	0.77	[0.08, 1.47]	0.030	0.0%	0.572
Immunoglobulin M	5	-0.05	[-0.53, 0.43]	0.844	0.0%	0.912
Withers height	12	0.06	[-0.19, 0.30]	0.664	0.0%	0.982
Heart girth	9	0.08	[-0.47, 0.62]	0.781	72.6%	< 0.001
Body length	5	-0.13	[-0.52, 0.26]	0.511	20.6%	0.283
Hip width	4	0.41	[-0.06, 0.88]	0.088	0.0%	0.965
Rumen pH	8	0.05	[-0.28, 0.38]	0.755	0.0%	0.998
Total VFA	5	0.28	[-0.20, 0.76]	0.254	20.6%	0.286
Acetate	7	0.27	[-0.07, 0.62]	0.124	0.0%	0.462
Butyrate	8	0.07	[-0.44, 0.58]	0.780	56.2%	0.025
Propionate	7	-0.07	[-0.41, 0.28]	0.706	36.3%	0.151
NH ₃	9	-0.27	[-0.54, 0.00]	0.051	0.0%	0.472

SMD: Standardized Mean Differences; CI, Confidence Intervals; I², statistics of heterogeneity; VFA, Volatile Fatty Acids; N: Represents the total number of treatment–control comparisons contributing to each outcome.

3.1 Average Daily Gain and Feed Efficiency

The effects of EO administration on ADG were pooled from a total of 26 comparisons. The overall pooled effect size was SMD = 0.40 with 95% CI = 0.14, 0.66, which is significant ($P = 0.003$), as shown in Table 2. A substantial level of heterogeneity was observed in the analysis of ADG, with an I² value of 59.3% ($P < 0.001$). This means that approximately 59.3% of the variability in the effect sizes was due to differences between studies, suggesting some inconsistency in the results between studies. Figure 2 displays forest plots depicting the impact of EO administration on pre-weaning ADG of Holstein calves. A statistically significant relationship was found between EO supplementation and average daily liveweight gain of pre-weaned Holstein calves ($P < 0.05$). A funnel plot was used to assess the presence of publication bias and (Figure 3).

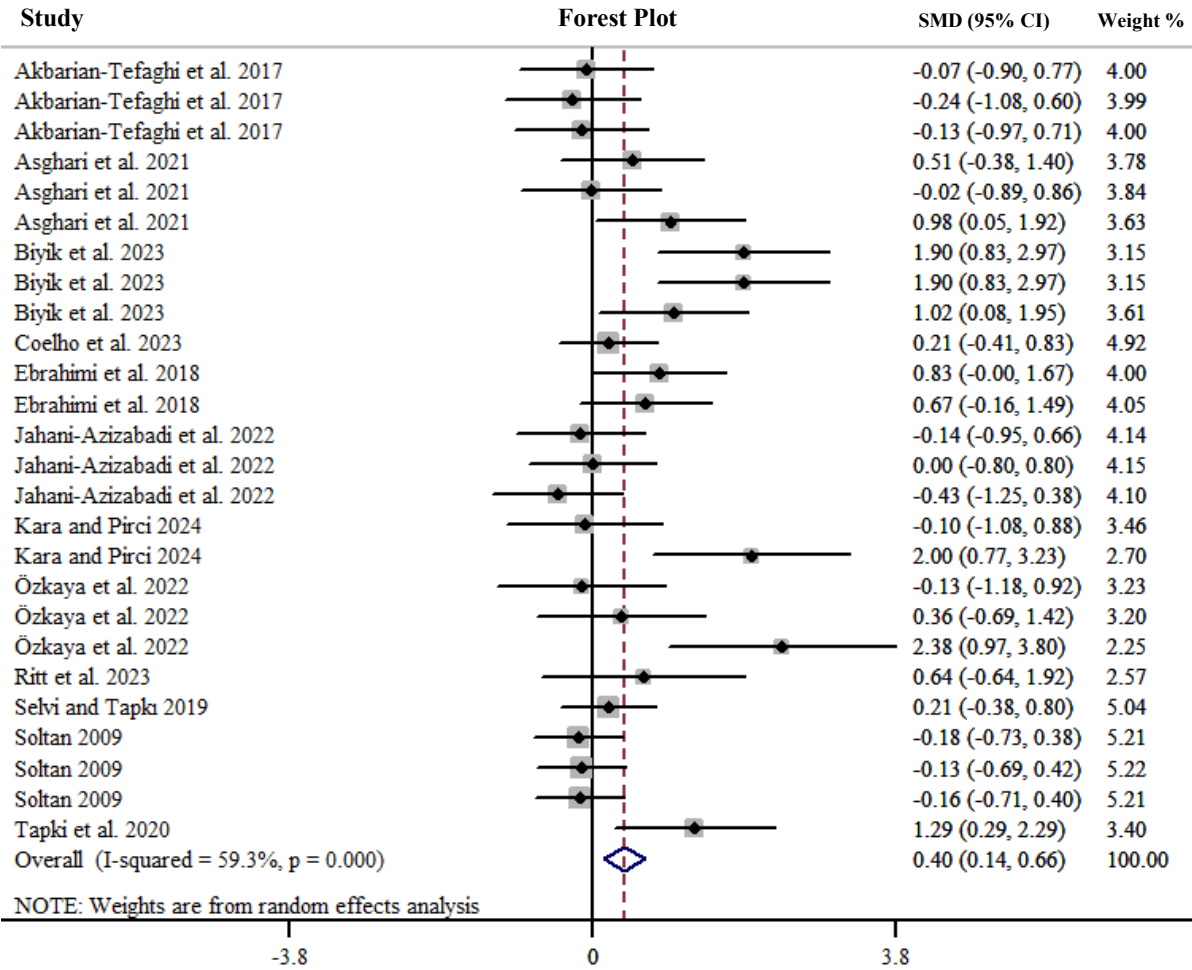


Figure 2. Forest Plot for Average Daily Gain of Holstein calves.

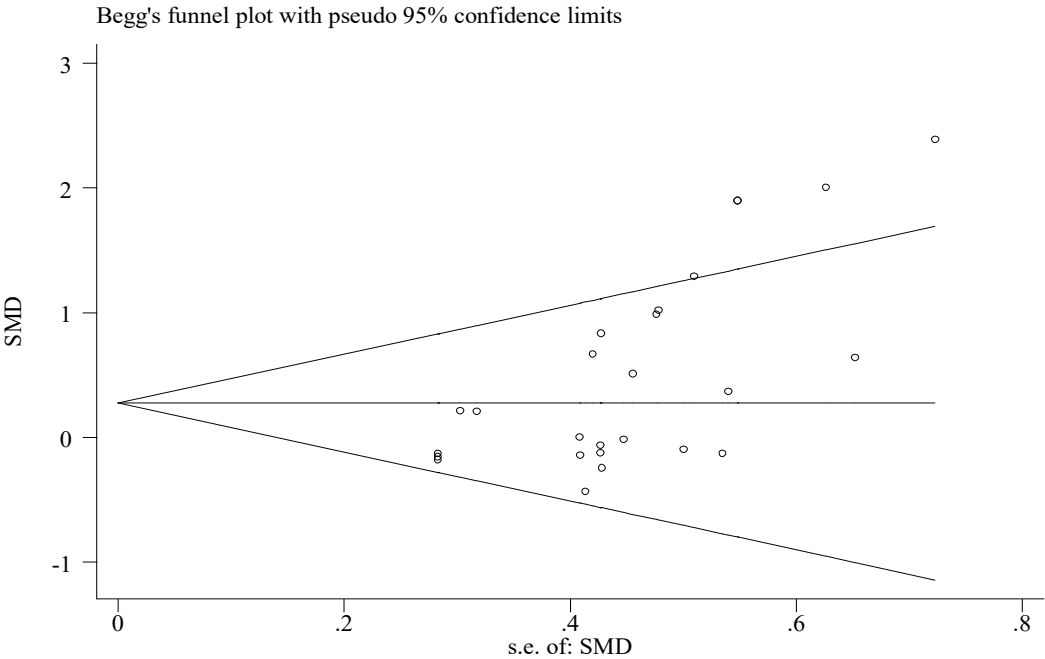


Figure 3. Funnel plot illustrating potential publication bias in studies assessing the impact of EO administration on average daily gain in Holstein calves.

3.2 Feed Efficiency

Meta-analysis was performed to investigate the effects of EO administration in pre-weaning dairy calves on feed efficiency (12 comparisons). The summarized results of the SMD and the 95% confidence interval (CI) for each study are shown in Table 2. The effect of EO administration on feed efficiency of dairy calves is shown in Figure 4 as forest plots. Feed efficiency was considerably higher in EO treated calves. However, the difference did not reach the significance level ($P = 0.089$). (SMD = 0.29; 95% -0.04, 0.49; $I^2 = 7.3\%$; $P = 0.375$). A funnel plot was used to assess the presence of publication bias (Figure 5) and it was determined that there was no publication bias ($P=0.403$).

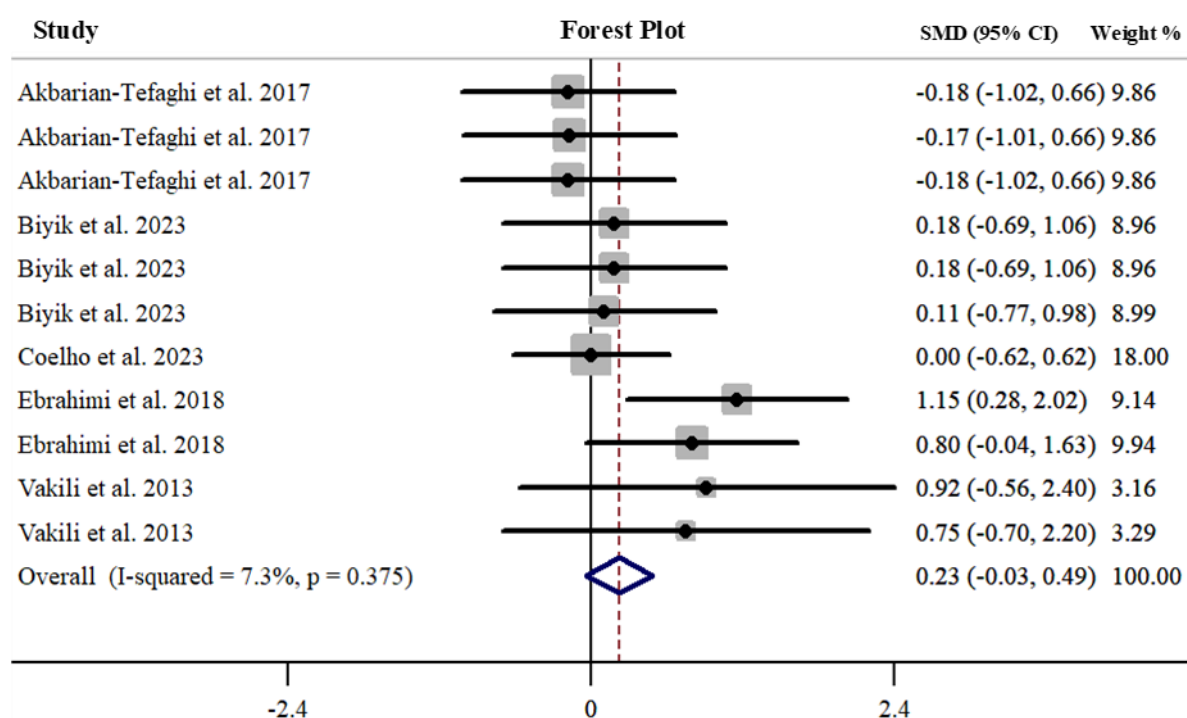


Figure 4. Forest Plot for Feed Efficiency of Holstein calves.

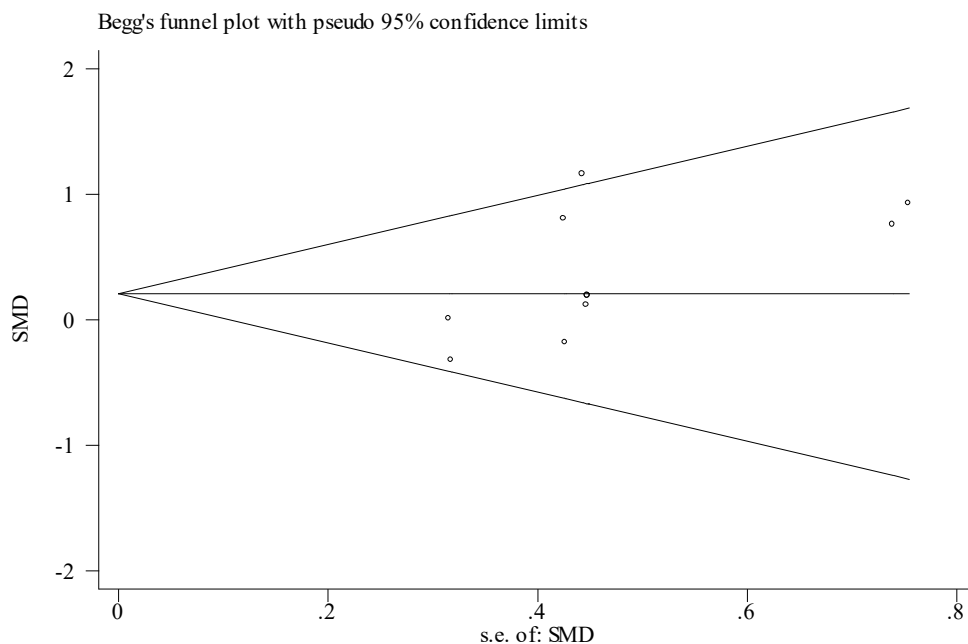


Figure 5. Funnel plots of the publication bias of the studies examining the effect of EO on Feed Efficiency of Holstein calves.

3.3 Fecal Score

A total of 10 comparisons were pooled for the effect of EO administration on fecal score in dairy calves. The overall pooled effect size was found to be $SMD = -0.34$ with 95% CIs = -0.73 , -0.06 . A substantial level of heterogeneity was observed in the analysis of fecal score, with an I^2 value of 55.5% ($P < 0.05$). This means that about 55.5% of the variation in effect size was due to differences in study results, suggesting some inconsistency in results between studies. Despite the notably lower fecal score in EO treated calves, this difference was not statistically significant ($P = 0.217$), and the considerable heterogeneity between the results could be the reason for this insignificant difference. The forest plot of the effect of EO administration on fecal score in dairy calves is shown in Figure 6. The presence of publication bias was assessed using a funnel plot in Figure 7 and analysis revealed no statistically significant publication bias ($P = 0.093$).

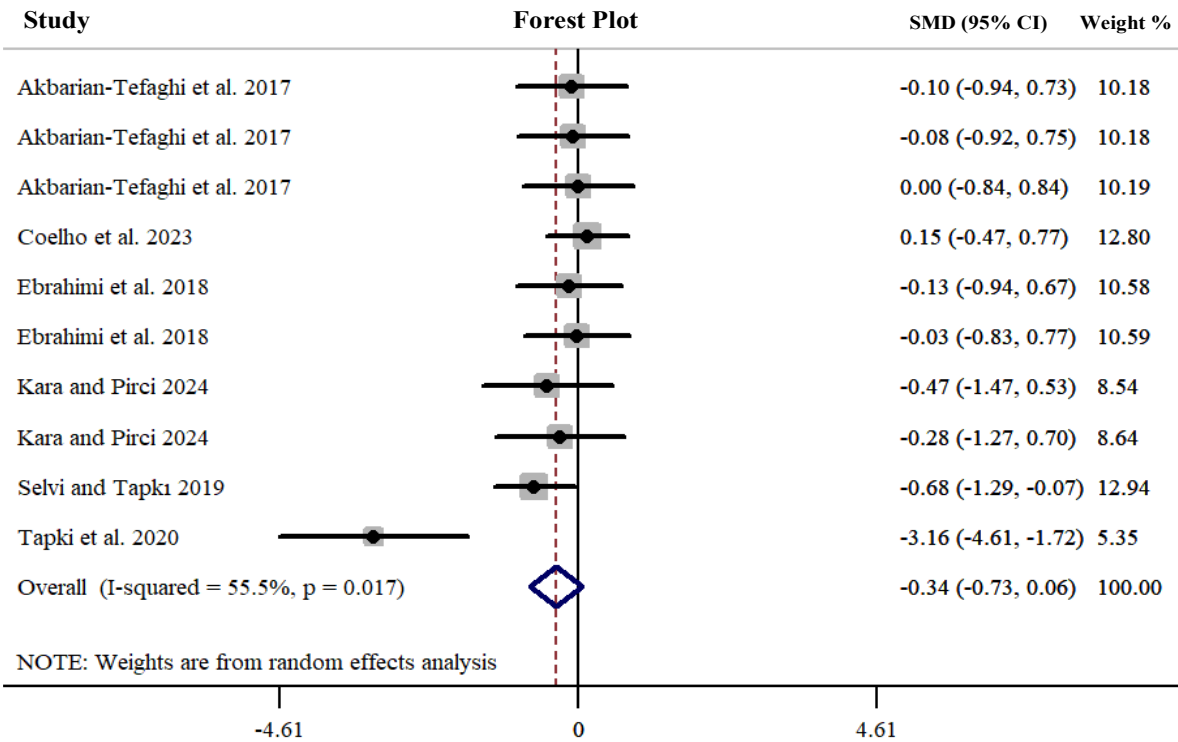


Figure 6. Forest Plot for Fecal Score of Holstein Calves.

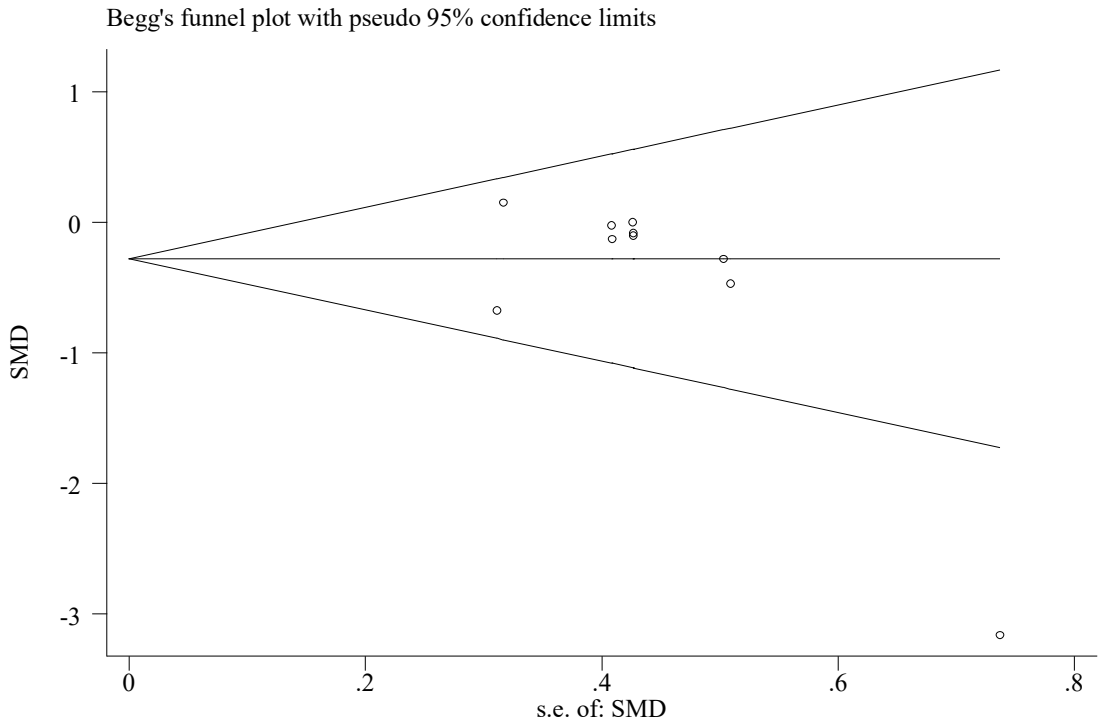


Figure 7. Funnel plots of the publication bias of the studies examining the effect of EO on Fecal Score of Holstein calves.

3.4 Immunoglobulins

A total of 11 comparisons were pooled for the effects of EO administration on serum IgG levels in dairy calves receiving EO until weaning. The total pooled effect size shown in Table 2 was SMD = 0.46 with 95% CI = 0.18, 0.75. Furthermore, heterogeneity was not observed in the analysis of IgG levels, with an I^2 value of 0.0% ($P > 0.05$). The effects of EO administration on IgG level of EO treated dairy calves at weaning is presented as forest plot in Figure 8. Meta-analysis results indicate that EO administration significantly increased serum IgG level of pre-weaned dairy calves ($P < 0.001$). The presence of publication bias was assessed using a funnel

plot in Figure 9, it was determined that there was no publication bias ($P > 0.05$)

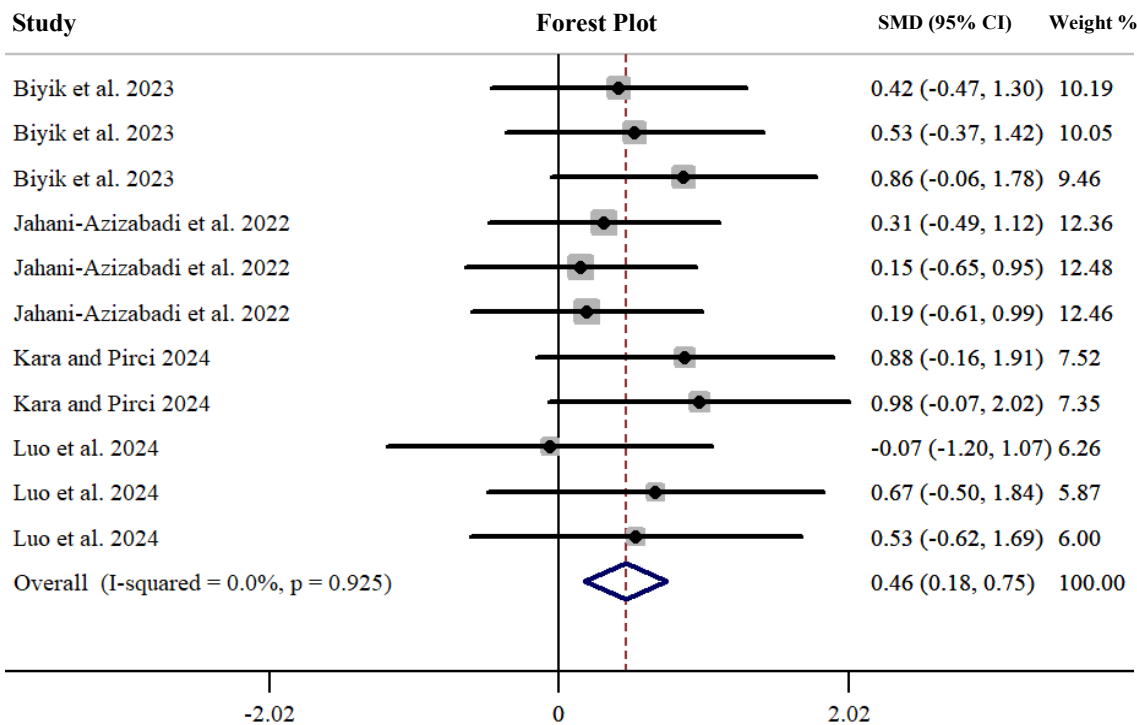


Figure 8. Forest Plot for Serum Immunoglobulin G levels of Holstein calves.

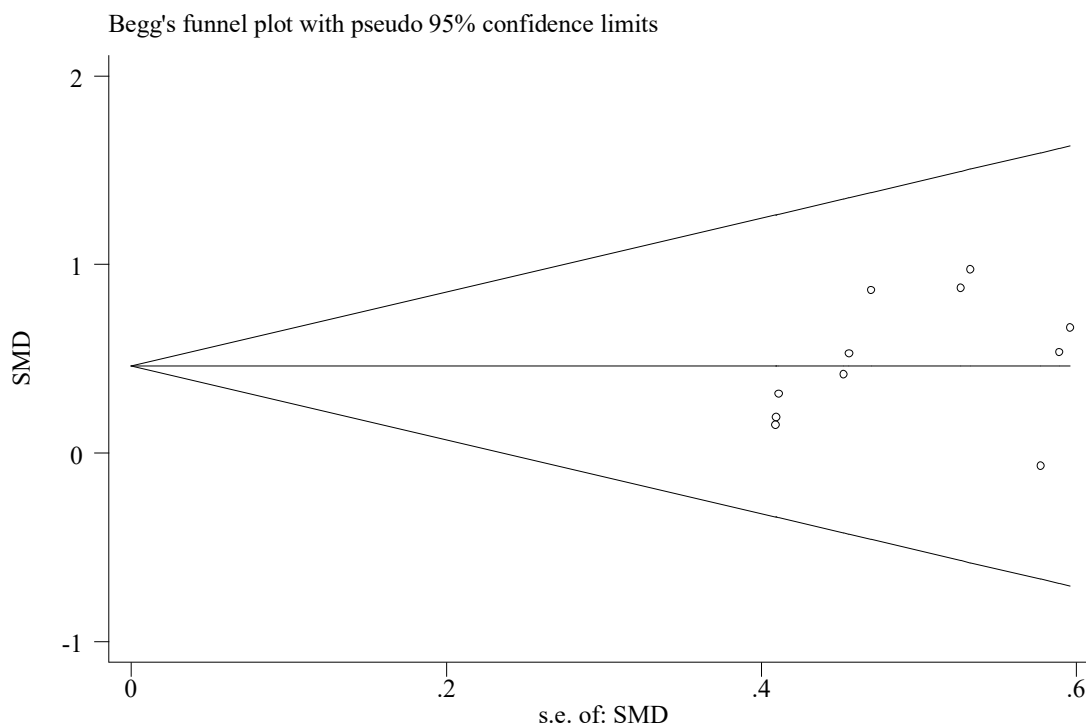


Figure 9. Funnel plots of the publication bias of the studies examining the effect of EO on Immunoglobulin G of Holstein calves.

The results of the SMD and the 95% CIs are shown in Table 2. Meta-analysis results revealed that EO supplementation significantly increased serum IgA levels in pre-weaning dairy calves compared to control group calves (SMD = 0.77; 95% CI: 0.08, 1.47; $P < 0.05$). EO supplementation was also evaluated for its impact on calf serum IgM levels at weaning (6 comparisons). The summarized results for the SMD and the 95% CI can be seen in Table 2. Results showed that EO administration had no significant influence on serum IgM levels in pre-weaned calves (SMD = -0.05; 95% CI: -0.53, 0.43; $I^2 = 0.0\%$). The high heterogeneity of the results may be responsible for this.

3.5 Ruminal Parameters

This study also examined the effects of essential oil supplementation on various rumen parameters in calves during the pre-weaning period. There was no correlation between EO supplementation and rumen pH of dairy calves at weaning (SMD = 0.05; 95% CI: -0.28, 0.38; $I^2 = 0.0\%$; $P = 0.998$). EO supplementation had no significant effect on total rumen VFA measured at weaning in dairy calves (SMD = 0.28; 95% CI: -0.20, 0.76; $I^2 = 20.6\%$). While rumen acetate concentrations were notably elevated (7 comparisons) with EO supplementation, the differences between the control and EO groups were not statistically significant (SMD = 0.27; 95% CI: -0.07, 0.62; $P = 0.124$; $I^2 = 17.6\%$). In addition, no significant difference was

observed in the rumen butyrate levels of the calves, the overall pooled effect size for butyrate levels was determined to be $SMD = 0.07$ with $95\% CI = -0.44, 0.58$ (Table 2). Similar results were also observed for the levels of ruminal propionate in calves before they were weaned. In pre-weaning Holstein calves, EO supplementation did not significantly affect ruminal propionate levels ($SMD = -0.07$; $95\% CI: -0.41, 0.28$; $I^2 = 36.3\%$). However, as shown in Figure 10, forest plots from studies investigating the effect of EO supplementation on rumen NH_3 concentrations in calves showed that EO supplementation significantly reduced NH_3 concentrations at weaning ($P < 0.05$), in the rumen of calves supplemented with EO until weaning as presented in Figure 10 ($SMD = -0.27$; $95\% CI: -0.54, 0.001$; $I^2 = 0.0\%$). The presence of publication bias was assessed using a funnel plot in Figure 11 and it was determined that there was no publication bias ($P > 0.05$).

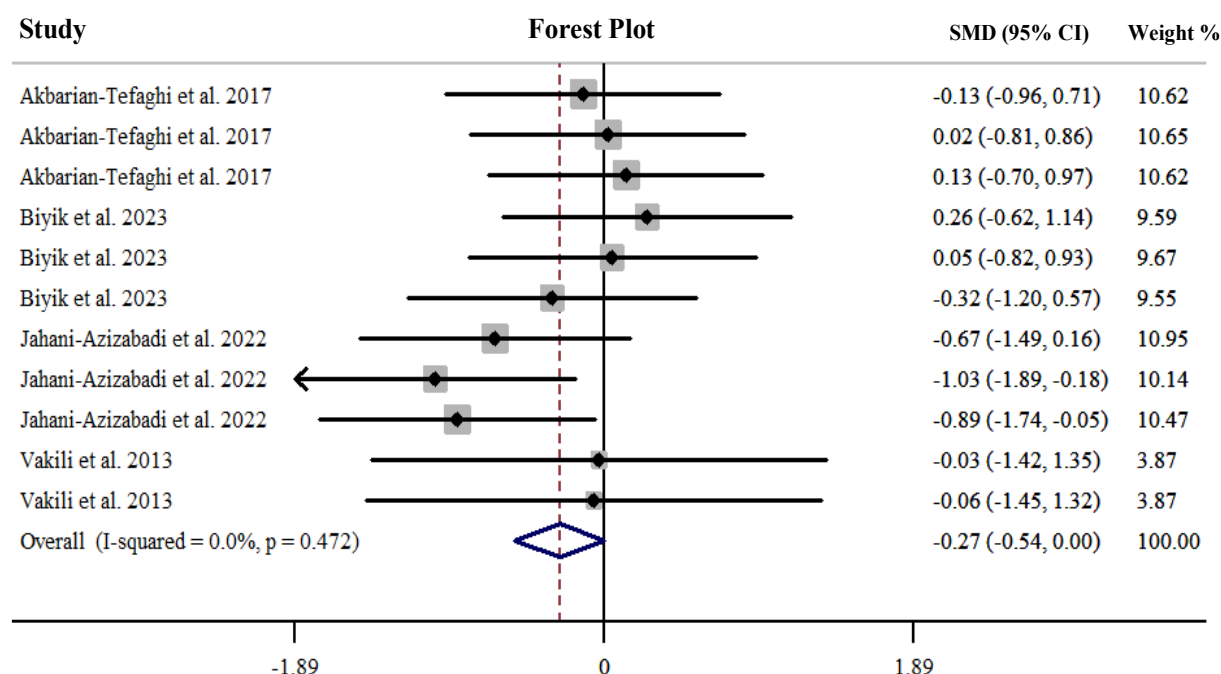


Figure 10. Forest Plot for rumen NH_3 concentration of Holstein calves.

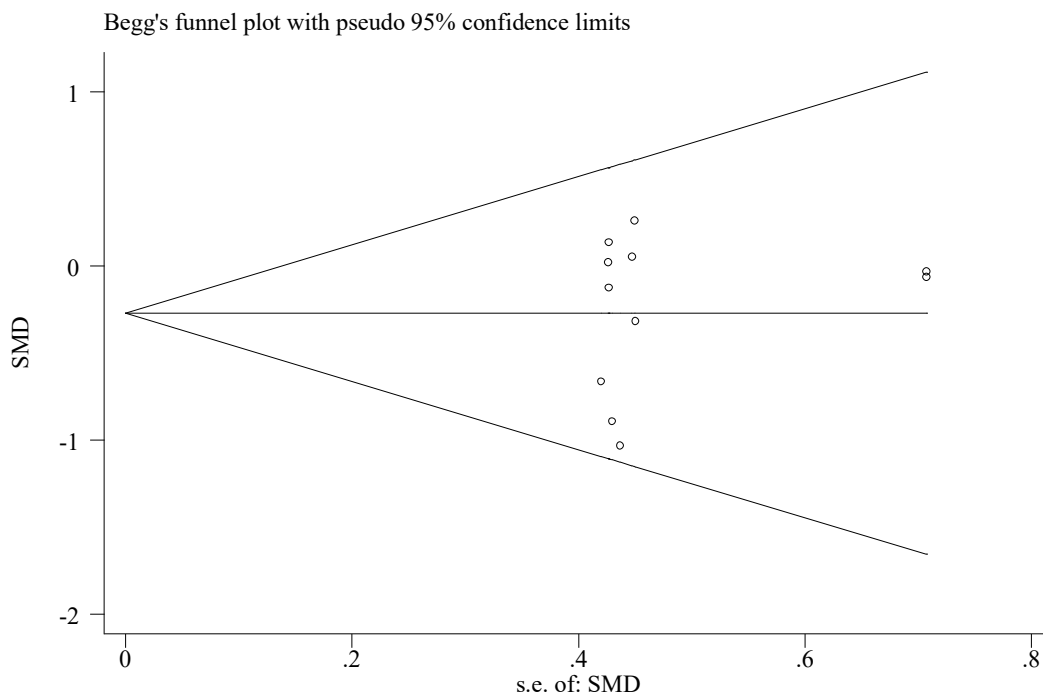


Figure 11. Funnel plots of the publication bias of the studies examining the effect of EO on NH_3 concentration of Holstein calves.

3.6 Body Measurements

The effects of EO supplementation weaning body measurements of the calves supplemented with EO in the pre-weaning period were analyzed. A total of 12 comparisons were pooled for the effects of EO treatment on weaning withers height measurements of dairy calves receiving EO in the pre-weaning period ($\text{SMD} = 0.06$; 95% CI = -0.19, 0.30). Despite EO supplemented calves had slightly higher withers height at weaning, the differences were not statistically significant ($P = 0.664$). Figure 12 shows the forest plot for withers height of Holstein calves. Similarly, EO supplementation had no significant effect on heart girth ($\text{SMD} = 0.77$; 95% CI: -0.47, 0.62; $I^2 = 72.6\%$) and body length ($\text{SMD} = -0.13$; 95% CI: -0.52, 0.26; $I^2 = 20.6\%$) measurements of calves received EO in pre-weaning period (Table 2). Conversely, hip width of the calves supplemented with EO was notably higher than nontreated calves. However, this difference did not reach the significance level ($P = 0.088$). The presence of publication bias was assessed using a funnel plot in Figure 13, it was determined that there was no publication bias ($P > 0.05$).

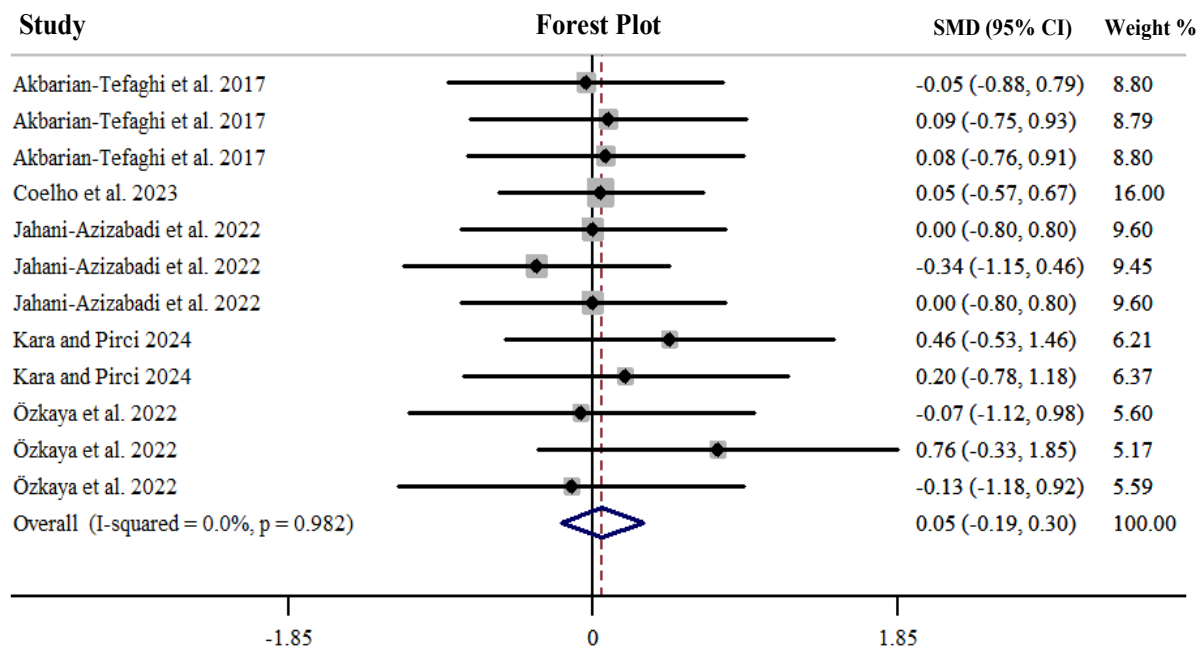


Figure 12. Forest Plot for Withers Height of Holstein Calves.

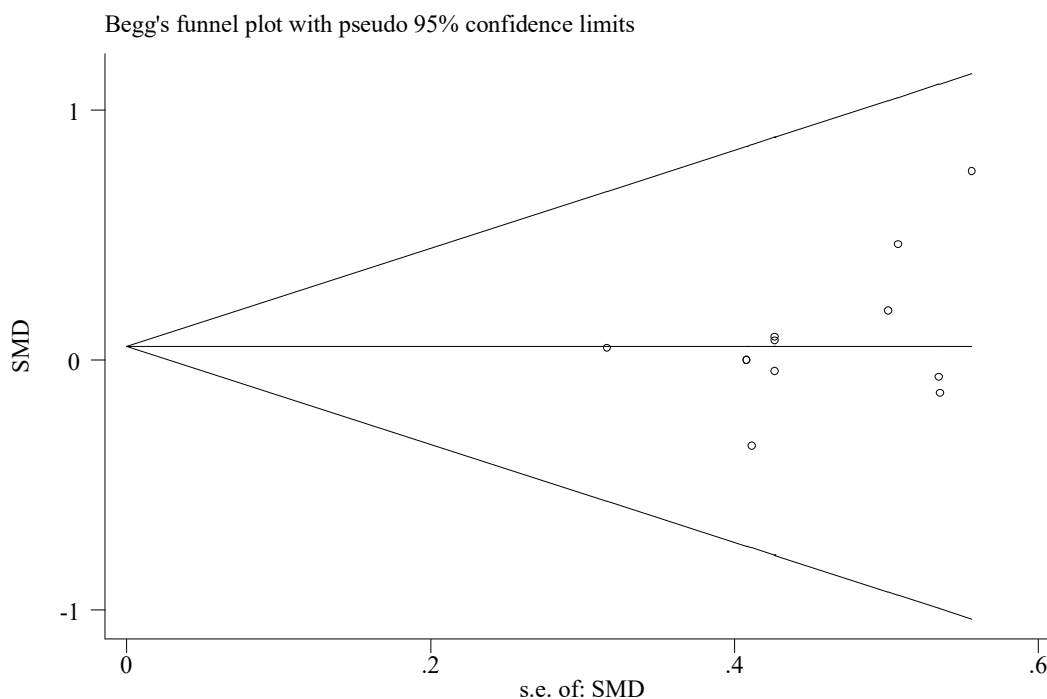


Figure 13. Funnel plots of the publication bias of the studies examining the effect of EO on Withers Height of Holstein calves.

4. Discussion

The increasing restrictions on antibiotic use in livestock production due to concerns regarding antimicrobial resistance and food residues have increased interest in alternative feed additives. Among these, essential oils (EOs) have been investigated for their potential effects on calf health, growth, development, and immunity (Özdemir *et al.*, 2022; Ritt *et al.*, 2023; Froehlich

et al., 2017; Wells, 2023). In the present meta-analysis, the effects of EO supplementation on growth performance, body development, fecal score, feed efficiency, immunity, and rumen parameters in pre-weaned dairy calves were evaluated. EO supplementation significantly increased ADG in pre-weaned calves. Several individual studies, including those evaluating oregano, *Salvia officinalis*, and rosemary EOs, reported relatively pronounced effects on ADG (Tapki *et al.*, 2020; Biyik *et al.*, 2023; Kara and Pirci, 2024). However, formal subgroup analyses would provide a more robust basis for comparing the effects of different EO sources, and the findings from individual studies should therefore be interpreted with caution. Although EO supplementation tended to improve feed efficiency, the pooled effect did not reach statistical significance ($P = 0.089$). This heterogeneity could be due to different calf rearing methods, where calves are fed high amounts of solid feed in some studies, but mostly milk in others.

Diarrhea is an important health problem in pre-weaned calves and may negatively affect growth performance (Hordofa *et al.*, 2021; Lora *et al.*, 2018; Schinwald *et al.*, 2022). In the present meta-analysis, EO supplementation reduced fecal scores in pre-weaned dairy calves; however, the pooled effect was not statistically significant. Although the absence of statistical significance limits the strength of the evidence, the direction of the pooled effect is biologically relevant because improved fecal consistency may indicate better gastrointestinal health and could potentially reduce the negative consequences of diarrhea on nutrient utilization and growth. The relatively high heterogeneity observed among the included comparisons ($I^2 = 55.5\%$) may have contributed to the variation in the observed responses. In particular, the oregano essential oil evaluated by Tapki *et al.* (2020) showed a pronounced reduction in fecal score, suggesting that differences among EO sources or experimental conditions may influence the magnitude of the response.

Immunoglobulins (Ig), or antibodies, are glycosylated proteins produced by plasma cells that play a key role in fighting disease agents (Megha and Mohanan 2021). Calves with low immunoglobulin levels are more susceptible to disease (Furman-Fratczak *et al.*, 2011). Serum immunoglobulin levels are very important for calf health, especially during the period of passive immunity and the transition from passive to active immunity. In the present meta-analysis, EO supplementation significantly increased serum IgG and IgA concentrations, whereas IgM was not significantly affected. The increase in IgG and IgA may have biological relevance because these immunoglobulins contribute to humoral immune defense, suggesting that EO supplementation may support the development of immune competence during the vulnerable pre-weaning period. All kinds of EO sources seems to be highly effective in

increasing IgG level of the pre-weaning calves as evident by the studies in presented in Figure 8. One of the major immune cells which produce antibodies, are B lymphocytes. (Rival *et al.*, 2024). Biyik *et al.* (2023) reported that EOs may contribute to increased IgG concentrations by stimulating B lymphocytes, possibly through modulation of cytokine production, enhancement of antigen presentation, or activation of signaling pathways involved in B-cell proliferation and differentiation. These immunomodulatory effects may contribute to improved immune responses in EO-treated calves and may partly explain the favorable effects observed on fecal score, growth performance, and feed efficiency. Overall, the findings suggest that EO supplementation may support immune function and enhance resistance to disease during the pre-weaning period, particularly during the transition from passive to active immunity. During the transition period from milk to solid feed, rumen development and VFAs play a critical role for calves. Propionate, acetate and butyrate are the primary VFAs produced by microbial fermentation of carbohydrates in the rumen (Liu *et al.*, 2020). These short-chain fatty acids also serve as an important source of energy for the calf. Butyrate, propionate, and acetate play important roles in rumen development and energy metabolism (Górka *et al.*, 2018; Wang *et al.*, 2023; Urrutia and Harvatine, 2017). In the present meta-analysis, EO supplementation did not significantly affect total ruminal VFA concentration. This finding indicates that EO supplementation did not consistently alter overall ruminal VFA production in pre-weaned calves. However, Biyik *et al.* (2023) reported an increase in total ruminal VFA following rosemary EO supplementation, suggesting that the effects of EO supplementation may vary depending on the EO source and experimental conditions. Similarly, there was no statistically significant differences on the acetate, butyrate and propionate levels in rumen liquid with EO supplementation of pre-weaning dairy calves. However, it was determined that the amount of rumen ammonia-N was significantly reduced with the EO structure. This reduction may be related to the antimicrobial properties of EOs and their potential effects on microorganisms involved in protein degradation and amino acid deamination (McIntosh *et al.*, 2003). Reducing the ammonia-N level in the rumen of ruminants improves protein structure, controls nitrogen waste, and allows for the maintenance of energy availability by directing it to more efficient functions such as fatty acid production under microbial heat. In general, in this case, as seen in other processes, the animal's characteristics and feed properties improve.

5. Conclusion

In conclusion, our findings indicate that EO supplementation may have positive effects on selected aspects of growth performance in pre-weaning dairy calves, particularly by increasing average daily gain. Although feed efficiency tended to improve, the pooled effect did not reach

statistical significance. EO supplementation also showed beneficial effects on selected immune parameters, particularly IgG and IgA, while its effects on other immune and performance-related outcomes were not consistently significant. Furthermore, EO supplementation significantly reduced ruminal ammonia-N levels, which may indicate a potential effect on ruminal nitrogen metabolism. Based on the results of this study, EOs may represent a potential natural feed additive for supporting the health and performance of dairy calves during the pre-weaning period. However, these effects were not consistent across all evaluated outcomes, and further well-designed studies using standardized EO sources, doses, and feeding conditions are needed to clarify the potential role of EOs in improving the health and performance of dairy calves before weaning.

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