SYSTEMATIC REVIEW

The use of essential oils as a growth promoter for small ruminants: a systematic review and meta-analysis [version 1; peer review: 2 approved]

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Abstract

**Background:** Due to their antimicrobial properties and safety, essential oils are currently proposed as a sustainable option for antibiotic alternatives in the livestock sector. This current systematic review and meta-analysis investigated the effects of dietary essential oil supplements on growth response of small ruminants.

**Methods:** A total of 12 studies (338 small ruminants) were included in this meta-analysis. The overall effect size was quantified using Hedges’ *g* with 95% confidence interval (CI) using a fixed-effect model. Publication bias was inspected using Begg’s and Egger’s tests, followed by trim and fill method to detect the number of potential missing studies.

**Results:** Insignificant heterogeneity among studies was detected both on dry matter intake (DMI; *P* of Q = 0.810; I-square = 0.00%), average daily gain (ADG; *P* of Q = 0.286; I-square = 17.61%), and feed conversion ratio (FCR; *P* of Q = 0.650; I-square = 0.00%). The overall effect size showed that essential oils supplementation had no significant impact on DMI (Hedges’ *g* = -0.12; 95% CI = -0.50 to 0.26; *P* = 0.429) and FCR (Hedges’ *g* = -0.17; 95% CI = -0.55 to 0.22; *P* = 0.284), but had a significant positive impact on ADG (Hedges’ *g* = 0.44; 95% CI = 0.12 to 0.76; *P* = 0.002). The result of publication bias analysis showed that DMI, ADG, and FCR did not present any significant biases (*P* > 0.10), and no potential missing studies detected.

**Conclusions:** Dietary essential oil could improve ADG of small ruminants, without any alteration on DMI and FCR. Further research in this topic is still required to provide stronger evidence of the potency of essential oil as a growth promoter for small ruminants.

**Keywords**

Antibiotics alternative, Average daily gain, Goats, Natural feed additives, Protozoa, Secondary metabolites, Sheep.
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Introduction
In animal nutrition, antibiotics become the first choice of feed additive due to their substantial benefit toward health and productivity. However, the routine use of this chemical additive yields residues in livestock products, and is also responsible for the development of microbial antibiotic resistance1−2. These factors represent a dangerous risk to human health, which has led to the global drive to reduce antibiotic use in the livestock sector. As a result, several natural products have been proposed to be used as antibiotic alternatives3−4.

Among natural feed additives, essential oils have a unique mechanism of action in livestock production. They can manipulate rumen fermentation characteristics5,6 and subsequently improve growth rate7−8. However, other findings showed no meaningful effect of this feed additive on productive performance9,10, while another study showed a negative impact11. The inconsistent results among studies requires an appropriate tool to quantitate the overall effect. Therefore, this study was conducted to measure the quantitative effects of dietary essential oil supplementation on the growth response of small ruminants using a systematic review and meta-analysis approach.

Methods
The systematic review and meta-analysis was conducted according to the Preferred Reporting Items for systematic Reviews and Meta-Analyses (PRISMA) guideline12. A completed PRISMA checklist is available in Reporting guidelines13.

Eligibility criteria
The inclusion and exclusion of the study were based on participants, interventions, comparisons, outcomes, and study design (PICOS) criteria as indicated in Table 1. Additionally, only publications written in English which was included in this study. All dates up until the date last searched were included.

Literature search strategy
The literature search was carried out using the following electronic databases: Scopus, PubMed, and SciELO. The search was last performed on 30 April 2020. Table 2 shows the full electronic search strategy.

| Table 1. PICOS criteria. |
|--------------------------|
| **Inclusion criteria**   | **Exclusion criteria** |
| Participants             | Sheep or goat          | Other species |
| Interventions            | Dietary essential oil supplementation | Irrelevant treatment |
| Comparisons              | Control group (basal diet only) | |
| Outcomes                 | DMI, ADG, and FCR      | No related outcome |
| Study design             | Randomized controlled in vivo trials | In vitro trials |

Table 2. Search strategy.

| Database | Search strategy |
|----------|-----------------|
| Scopus   | (TITLE (oil) AND TITLE (growth OR performance) AND TITLE (sheep OR goat OR lamb OR kid)) |
| PubMed   | ((ti:(Title)) AND (growth[Title] OR performance[Title])) AND (sheep[Title] OR goat[Title] OR lamb[Title] OR kid[Title]) |
| SciELO   | (ti:(oil)) AND (ti:(growth OR performance)) AND (ti:(sheep OR goat OR lamb OR kid)) |

Results from the search were firstly checked for duplicates. After duplicate studies were removed, F.A. and A.N.H. screened titles and abstracts independently using the eligibility criteria (Table 1). Full texts of the selected studies were then further examined to find eligible studies. In the case of any disagreement, this was resolved by adjudication from M.M. The authors of the included studies were not contacted for further clarification.

Effect size quantification
The overall effect size was quantified using Hedges’ g16 using a fixed-effect model. This model was chosen due to the insignificant heterogeneity among studies after checked using Cochran’s Q17 and I-square18.

Publication bias analysis
Publication bias was inspected using Begg’s19 and Egger’s tests20, with P <0.10 set to determine the existence of publication bias. The trim and fill method21 was employed to detect the number of potential missing studies and to adjust the overall effect size. All meta-analysis procedures were performed using Meta-Essentials version 1.422.

Results
Figure 1 shows the PRISMA flow diagram. A total of 137 records were identified through database searching. Of these, 12 studies were eligible for the current meta-analysis. The essential oil sources included oregano1,22−24, thyme25,26, chavil27, juniper28, and mixed product29,30. Unfortunately, one study did not define the source of essential oil15. The main characteristics of the included studies are shown in Table 3. Extracted data of outcome measures is available as Extended data31.
Table 3. Main characteristics of the studies included in the meta-analysis.

| Authors               | Species | n  | EO source | Response variables     |
|-----------------------|---------|-----|-----------|------------------------|
| Aydin et al.          | Sheep   | 18  | Oregano   | DMI, ADG, FCR          |
| Ribeiro et al.        | Sheep   | 40  | Thyme     | DMI, ADG               |
| Lei et al.            | Goats   | 45  | NI        | ADG                    |
| Parvar et al.         | Sheep   | 40  | Chavil    | DMI, ADG, FCR          |
| Canbolat et al.       | Sheep   | 40  | Oregano   | DMI, ADG, FCR          |
| Yesilbag et al.       | Goats   | 18  | Juniper   | ADG                    |
| Gümüş et al.          | Sheep   | 24  | Oregano   | ADG                    |
| Baytok et al.         | Sheep   | 15  | Thyme     | ADG, FCR               |
| Malekhhahi et al.     | Sheep   | 10  | Mix A     | DMI, FCR               |
| Özdoğan et al.        | Sheep   | 20  | Mix B     | DMI, ADG, FCR          |
| Canbolat and Karabulut| Sheep   | 48  | Oregano   | ADG                    |
| Chaves et al.         | Sheep   | 20  | Juniper   | DMI, ADG, FCR          |

n: number of experimental animals; EO: essential oil; NI: no information; Mix A: a mixture of thymol, carvacrol, eugenol, limonene, and cinnamaldehyde EO; Mix B: a mixture of thyme leaf, daphne leaf, sage tea leaf, fennel seed, orange cortes, and myrtle leaf EO; DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.
Data of ADG from two studies\textsuperscript{11,25} were considered as outliers because their standardized residual was >3 and thus were excluded from effect size quantification. Insignificant heterogeneity among studies was detected both for DMI (\(P = 0.810\); I-square = 0.00\%), ADG (\(P = 0.286\); I-square = 17.61\%), and FCR (\(P = 0.650\); I-square = 0.00\%). As can be seen in Figure 2, the overall effect size showed that essential oil supplementation had no significant impact on DMI (\(P = 0.429\)) and FCR (\(P = 0.284\)), but had a significant positive impact on ADG (\(P = 0.002\)). The result of publication bias analysis showed that DMI, ADG, and FCR did not present any significant biases (\(P > 0.10\)) (Table 4). The trim and fill method also did not detect any potential missing studies for all parameters.

**Discussion**

The current meta-analysis showed that dietary essential oils significantly increased ADG of small ruminants. This finding probably related to the antimicrobial activity of essential oils, which could reduce ruminal protozoa population\textsuperscript{31,32}. Protozoa population may represent up to 50\% of the total biomass of rumen microbes\textsuperscript{33}. They have a negative impact on nitrogen utilization by ruminants because they engulf and digest bacteria,

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Forest plot of the effect of essential oil supplementation on growth response of small ruminants. DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.}
\end{figure}
thus reducing microbial protein flow to abomasum. Additionally, the presence of protozoa is also associated with methane production, which is responsible for the loss of up to 12% of gross energy intake by ruminants. Thereby, the reduction of the ruminal protozoa population by essential oil could increase microbial protein, as well as energy supply, which ultimately could improve the growth rate of small ruminants.

This study provides insight of the potency of essential oil as a growth promoter for small ruminants. However, the current findings should be interpreted with caution due to the limited data available. Moreover, the literature search only covers published literature, which could lead to publication bias. For that reason, further research in this topic is highly encouraged to provide stronger evidence.

Conclusions
The current meta-analysis reveals that dietary essential oil could improve average daily gain of small ruminants, without any alteration on dry matter intake and feed conversion ratio. However, further research in this topic is still highly recommended to provide more robust evidence.

Data availability
Underlying data
All data underlying the results are available as part of the article and no additional source data are required.

Extended data
Figshare: Extended data for ‘The use of essential oils as a growth promoter for small ruminants: a systematic review and meta-analysis’. https://doi.org/10.6084/m9.figshare.12298913.v3.

This project contains extracted data of outcome measures (dry matter intake, average daily gain, and feed conversion ratio).

Reporting guidelines
Figshare: PRISMA checklist for ‘The use of essential oils as a growth promoter for small ruminants: a systematic review and meta-analysis’. https://doi.org/10.6084/m9.figshare.12298034.v2.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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Table 4. Summary of publication bias analysis of the effect of dietary essential oil intervention on growth response of small ruminants.

| Parameters | P of Begg’s test | P of Egger’s test | Missing studies |
|------------|------------------|------------------|----------------|
| DMI        | 0.652            | 0.879            | 0              |
| ADG        | 1.000            | 0.605            | 0              |
| FCR        | 0.652            | 0.463            | 0              |

DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.
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The main purpose of this paper tries to investigate whether essential oils have any positive or negative effects on growth performance, such as dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR). The results shown that the essential oils provided for sheep and goats in 12 studies. Most experiments shown the essential oils can improve the above performance. The present study used the Begg's and Egger's tests which were meta-analysis procedures. Please indicate the original words and then can put abbreviations. It is not necessary to point out the authors' duties in study selection and data extraction. The authors need to make clarification from the literature on why the essential oils can be used as a growth promoter for small ruminants.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Agricultural production; Aquaculture production; Ecologic system; Conservation; Rural education and extension; Reproduction of animal (ex AI for animals); Nutrition on poultry, pigs, ...
cattle, sheep, goat, dog and cat, etc.; Management for animal production; Animal house design and arrangement; Animal behavior and welfare; Feed processing; Animal waste management; Extension education.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Linde du Toit
Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa

In this article the researchers conducted a meta analysis on the effects of essential oils on production parameters of small ruminants.

This is a well written article. The objective and methods employed are suitable and clearly defined within the text. The authors could have included the experimental design in the selection criteria as well as the type of rations used in the various studies. The researchers could have broadened the criteria to include more studies in the Meta analysis. Less than 20 studies were included but the authors did discuss this and the need for further research in their discussion. The quality of the article is acceptable for indexing.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Small ruminant nutrition and livestock GHG emissions

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