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.
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 alternatives14.

Among natural feed additives, essential oils have a unique mechanism of action in livestock production. They can manipulate rumen fermentation characteristics3,6 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 quantify 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-Analysis (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  | Irrelevant treatment     |
|                          | supplementation         | only                     |
| Comparisons              | Control group (basal    |                         |
|                          | diet only               |                         |
| Outcomes                 | DMI, ADG, and FCR       | No related outcome       |
| Study design             | Randomized controlled   | In vitro trials          |
|                          | in vivo 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     | ((oil>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)) |

Study selection

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.

Data extraction

Data extraction was performed independently by F.A. and A.N.H. The senior investigator (M.M.) solved any disagreements by discussion. Data extracted included the following items: 1) authors; 2) animal species; 3) number of animals; 4) essential oil source; and 5) growth response variables. Growth response variables consisted of dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR). Standard error or standard error of means were converted into standard deviation14. The data was pooled when a study used more than one dose of essential oils or tested both sexes of experimental animals15.

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 Q16 and I-square17.

Publication bias analysis

Publication bias was inspected using Begg’s18 and Egger’s tests19, with P <0.10 set to determine the existence of publication bias. The trim and fill method20 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 1.421.

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 oregano1,2,24, thyme1,25,26, chavil27, juniper1,28, and mixed product29. Unfortunately, one study did not define the source of essential oil3. The main characteristics of the included studies are shown in Table 3. Extracted data of outcome measures is available as Extended data30.
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           |
| Malekkihi 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 ($P$ of $Q = 0.810$; I-square = 0.00%), ADG ($P$ of $Q = 0.286$; I-square = 17.61%), and FCR ($P$ of $Q = 0.650$; 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.
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.

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).

References

1. Ben Y, Fu C, Hu M, et al.: Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ Res. 2019; 169: 483–493. [PubMed Abstract] [Publisher Full Text]
2. He Y, Yuan Q, Mathieu J, et al.: Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. npj Clean Water. 2020; 3(1): 4. [Publisher Full Text]
3. Oh J, Hristov AN: Effects of plant-derived bio-active compounds on rumen fermentation, nutrient utilization, immune response, and productivity of ruminant animals. ACS Symp Ser. 2016; 1218: 167–186. [Publisher Full Text]
4. Dhanasekaran DK, Dias-Silva TP, Filho ALA, et al.: Plants extract and bioactive compounds on rumen methanogenesis. Agrosyst Syst. 2019; 6. [Publisher Full Text]
5. Lin B, Lu Y, Salem AZM, et al.: Effects of essential oil combinations on sheep ruminal fermentation and digestibility of a diet with fumarate included. Anim Feed Sci Technol. 2013; 184(1–4): 24–32. [Publisher Full Text]
6. Poudel P, Froehlich K, Casper DP, et al.: Feeding essential oils to neonatal holstein dairy calves results in increased ruminal prevotellaceae abundance and propionate concentrations. Microorganisms. 2019; 7(5): 120. [PubMed Abstract] [Publisher Full Text] [Free Full Text]
7. Chaves AV, Stanford K, Dugan MER, et al.: Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livest Sci. 2008; 117(2–3): 215–224. [Publisher Full Text]
8. Lei Z, Zhang K, Li C, et al.: Dietary supplementation with Essential-oils-cobalt for improving growth performance, meat quality and skin cell capacity of goats. Sci Rep. 2018; 8(1): 11634. [PubMed Abstract] [Publisher Full Text] [Free Full Text]
9. Malekkhahi M, Tahmasbi AM, Naserian AA, et al.: Effects of essential oils, yeast culture and malate on rumen fermentation, blood metabolites, growth performance and nutrient digestibility of Baluchi lambs fed high-concentrate diets. J Anim Physiol Anim Nutr (Berl). 2015; 99(2): 221–229. [PubMed Abstract] [Publisher Full Text]
10. Pukrop JR, Campbell BT, Schoonmaker JP: Effect of essential oils on performance, liver abscesses, carcass characteristics and meat quality in feedlot steers. Anim Feed Sci Technol. 2019; 257: 114296. [Publisher Full Text]
11. Canbolat O, Filya I, Kamalak A: Effect of oregano oil on growth performance, rumen fermentation parameters and blood metabolites of growing lambs. Livest Res Rural Dev. 2018; 30(4): 59. [Reference Source]
12. Moher D, Liberati A, Tetzlaff J, et al.: Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(7): e1000097. Published Abstract | Publisher Full Text | Free Full Text

13. Andr F, Huda AN, Marjuki M: PRISMA checklist for 'The use of essential oils as a growth promoter for small ruminants: a systematic review and meta-analysis'. 2020. http://www.doi.org/10.6084/m9.figshare.12298034.v2

14. Greg JD, Wadke L, Wilhelm B, et al.: The efficacy of interventions applied during primary processing on contamination of beef carcasses with Escherichia coli: A systematic review-meta-analysis of the published research. Food Control. 2012; 27(2): 385–397. Publisher Full Text

15. Higgins JPT, Li T, Deeks JJ: Choosing effect measures and computing estimates of effect. In: Cochrane Handbook for Systematic Reviews of Interventions. 2019: 143–176. Publisher Full Text

16. Hedges LV, Oken I: Statistical Methods for Meta-Analysis. San Diego, CA USA: Academic Press; 1985.

17. Higgins JPT, Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21(11): 1539–1558. Published Abstract | Publisher Full Text

18. Begg CB, Mazumdar M: Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994; 50(4): 1088–1101. Published Abstract | Publisher Full Text

19. Egger M, Smith GD, Schneider M, et al.: Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315(7109): 629–34. Published Abstract | Publisher Full Text | Free Full Text

20. Duval S, Tweedie R: A nonparametric “trim and fill” method of accounting for publication bias in meta-analysis. J Am Stat Assoc. 2000; 95(449): 89–98. Publisher Full Text

21. Suurmond R, van Rhoe H, Hak T: Introduction, comparison, and validation of Meta-Essentials: A free and simple tool for meta-analysis. Res Synth Methods. 2017; 8(4): 537–553. Published Abstract | Publisher Full Text | Free Full Text

22. Canbolat Ö, Kanbabul A: Effect of urea and oregano oil supplementation on growth performance and carcass characteristics of lamb fed diets containing different amounts of energy and protein. Turk J Vet Anim Sci. 2010; 34(2): 119–128. Publisher Full Text

23. Gümüþ R, Erol HS, İmik H, et al.: The effects of the supplementation of lamb rations with oregano essential oil on the performance, some blood parameters and antioxidant metabolism in meat and liver tissues. Kafrkas Univ Vet Fak Derg. 2017; 23(3): 395–401. Publisher Full Text

24. Aydin ÖD, Merhan O, Yıldız G, et al.: The effect of oregano oil (Origanum vulgare) on the fattening performance and blood oxidant-antioxidant balance in post-weaned tuj lambs. Kafrkas Univ Vet Fak Derg. 2020; 26(1): 59–64. Publisher Full Text

25. Baytok E, Kara K, Aksu T, et al.: The effect of Mediterranean thyme (Thymbra spicata L., var. spicata) essential oil on fattening performance and ruminal parameters in lamb. J Anim Feed Sci. 2017; 26(4): 319–325. Publisher Full Text

26. Ribeiro ADB, Ferraz Junior MVC, Polizel DM, et al.: Thyme essential oil for sheep: Effect on rumen fermentation, nutrient digestibility, nitrogen metabolism, and growth. Arg Bras Med Vet e Zootec. 2019; 71(6): 2065–2074. Publisher Full Text

27. Panvar R, Ghoorchi T, Kasheh H, et al.: Effect of Ferulago angulata (Chavil) essential oil supplementation on lamb growth performance and meat quality characteristics, Small Rumin Res. 2018; 167: 48–54. Publisher Full Text

28. Yesilbag D, Binicik H, Celin I, et al.: Effects of juniper essential oil on growth performance, some rumen protozoa, rumen fermentation and antioxidant blood enzyme parameters of growing Saanen kids. J Anim Physiol Anim Nutr (Berl). 2017; 101(1): e67–76. Publisher Full Text

29. Özdoğan M, Önenç SS, Önenç A: Fattening performance, blood parameters and slaughter traits of Karya lambs consuming blend of essential oil compounds. African J Biotechnol. 2011; 10(34): 6663–6668. Publisher Full Text

30. Andr F, Huda AN, Marjuki M: Extended data for ‘The use of essential oils as a growth promoter for small ruminants: a systematic review and meta-analysis’. 2020. http://www.doi.org/10.6084/m9.figshare.12298913.v3

31. Patra AK, Yu Z: Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl Environ Microbiol. 2012; 78(12): 4271–4280. Published Abstract | Publisher Full Text | Free Full Text

32. Soltan YA, Natel AB, Araujo RC, et al.: Progressive adaptation of sheep to a microencapsulated blend of essential oils: Rumen fermentation, methane emission, nutrient digestibility, and microbial protein synthesis. Anim Feed Sci Technol. 2018; 237: 8–18. Publisher Full Text

33. Sylvestre JT, Kamati SKR, Yu Z, et al.: Evaluation of a real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal nitrogen. J Dairy Sci. 2005; 88(6): 2083–2095. Publisher Full Text

34. Newbold CJ, De la Fuente G, Belanche A, et al.: The role of ciliate protozoa in the rumen. Front Microbiol. 2015; 6: 1–14. Published Abstract | Publisher Full Text | Free Full Text

35. Wanapat M, Cherdhong A, Phetsackha K, et al.: Dietary sources and their effects on animal production and environmental sustainability. Anim Nutr. 2015; 1(3): 96–103. PubMed Abstract | Publisher Full Text | Free Full Text
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.**

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**Author Response 20 Aug 2020**

**Faizal Andri**, Gadjah Mada University, Yogyakarta, Indonesia

We would like to thank the reviewer for his helpful comments and suggestions. Please see our responses and changes as detailed below.

1. *Please indicate the original words and then can put abbreviations.*
   We have made sure that the original words have indicated before using abbreviations.

2. *It is not necessary to point out the authors' duties in study selection and data extraction.*
   We have deleted the statement regarding the authors' duties (see Study Selection and Data Extraction sections).

3. *The authors need to make clarification from the literature on why the essential oils can be used as a growth promoter for small ruminants.*
   We have included the clarification about why essential oils can be used as a growth promoter for small ruminants (see Discussion section).

**Competing Interests:** No competing interests were disclosed.

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**Reviewer Report 17 June 2020**

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**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 s 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

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.

Author Response 20 Aug 2020
Faizal Andri, Gadjah Mada University, Yogyakarta, Indonesia

We are grateful to the reviewer for his valuable comments and suggestions. Please see our responses and changes as detailed below.

1. 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 use of experimental design and type of ration as selection criteria will left only a small number of eligible studies for synthesis, therefore we do not consider these elements as selection criteria. However, we showed these information as additional study characteristics (see Table 3).

2. 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 studies in this aspect is currently still very limited and we have addressed this issue (see Discussion section).

Competing Interests: No competing interests were disclosed
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