## SYSTEMATIC REVIEW



# **REVISED** The use of essential oils as a growth promoter for small

# ruminants: a systematic review and meta-analysis [version 2;

# 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 dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) 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 DMI (*P* of Q = 0.810; I-square = 0.00%), ADG (*P* of Q = 0.286; Isquare = 17.61%), and 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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Any reports and responses or comments on the article can be found at the end of the article.

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#### **REVISED** Amendments from Version 1

The manuscript was revised according to the comments from reviewers with the following details:

1. Indicated original words before using abbreviations.

- 2. Deleted the statement regarding the authors' duties in study selection and data extraction.
- 3. Included the discussion about why essential oils can be used as a growth promoter for small ruminants

4. Added data about the type of ration and experimental design in Table 3

Any further responses from the reviewers can be found at the end of the article

#### 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 resistance<sup>1,2</sup>. 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 alternatives<sup>3,4</sup>.

Among natural feed additives, essential oils have a unique mechanism of action in livestock production. They can manipulate rumen fermentation characteristics<sup>5,6</sup> and subsequently improve growth rate<sup>7,8</sup>. However, other findings showed no meaningful effect of this feed additive on productive performance<sup>9,10</sup>, while another study showed a negative impact<sup>11</sup>. 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 dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) 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) guideline<sup>12</sup>. A completed PRISMA checklist is available in *Reporting guidelines*<sup>13</sup>.

#### **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.

#### Study selection

Results from the search were firstly checked for duplicates. After duplicate studies were removed, the titles and

### Table 1. PICOS criteria.

	Inclusion criteria	<b>Exclusion criteria</b>
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 <i>in vivo</i> trials	<i>In vitro</i> trials

DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.

#### 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))

abstracts were screened using the eligibility criteria (Table 1). Full texts of the selected studies were then further examined to find eligible studies. The authors of the included studies were not contacted for further clarification.

#### Data extraction

Data extracted included the following items: 1) authors; 2) animal species; 3) number of animals; 4) type of ration; 5) essential oil source; 6) experimental design, and 7) growth response variables. Growth response variables consisted of DMI, ADG, and FCR. Standard error or standard error of means were converted into standard deviation<sup>14</sup>. The data was pooled when a study used more than one dose of essential oils or tested both sexes of experimental animals<sup>15</sup>.

#### Effect size quantification

The overall effect size was quantified using Hedges'  $g^{16}$  using a fixed-effect model. This model was chosen due to the insignificant heterogeneity among studies after checked using Cochran's Q<sup>16</sup> and I-square<sup>17</sup>.

### Publication bias analysis

Publication bias was inspected using Begg's<sup>18</sup> and Egger's tests<sup>19</sup>, with P < 0.10 set to determine the existence of publication bias. The trim and fill method<sup>20</sup> 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 1.4<sup>21</sup>.

### Results

Figure 1 shows the PRISMA flow diagram. A total of 137 records were identified through database searching. Of these, 12



Figure 1. PRISMA flow diagram.

studies were eligible for the current meta-analysis. The essential oil sources included oregano<sup>11,22–24</sup>, thyme<sup>25,26</sup>, chavil<sup>27</sup>, juniper<sup>7,28</sup>, and mixed product<sup>9,29</sup>. Unfortunately, one study did not define the source of essential oil<sup>8</sup>. The main characteristics of the included studies are shown in Table 3. Extracted data of outcome measures is available as *Extended data*<sup>30</sup>.

Data of ADG from two studies<sup>11,25</sup> 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<sup>31,32</sup>. Protozoa population may represent up to 50% of the total biomass of rumen microbes<sup>33</sup>. They have a negative impact on nitrogen utilization by ruminants because they engulf and digest bacteria, thus reducing microbial protein flow to abomasum<sup>34</sup>. 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<sup>35</sup>. 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 ADG of small ruminants, without any alteration

Authors	Species	n	Type of ration	EO source	Experimental design	Response variables
Aydin <i>et al.</i> <sup>24</sup>	Sheep	18	Pasture grass + CF	Oregano	CRD	DMI, ADG, FCR
Ribeiro <i>et al.</i> <sup>26</sup>	Sheep	40	TMR	Thyme	RBD	DMI, ADG
Lei <i>et al.</i> <sup>8</sup>	Goats	45	TMR	NI	CRD	ADG
Parvar et al. <sup>27</sup>	Sheep	40	TMR	Chavil	CRD	DMI, ADG, FCR
Canbolat <i>et al</i> . <sup>11</sup>	Sheep	40	TMR	Oregano	CRD	DMI, ADG, FCR
Yesilbag <i>et al.</i> <sup>28</sup>	Goats	18	TMR	Juniper	CRD	ADG
Gümüş et al.23	Sheep	24	Wheat straw + CF	Oregano	CRD	ADG
Baytok <i>et al</i> . <sup>25</sup>	Sheep	15	Lucerne hay + CF	Thyme	RBD	ADG, FCR
Malekkhahi <i>et al</i> .9	Sheep	10	TMR	Mix A	CRD	DMI, FCR
Özdoğan <i>et al.</i> <sup>29</sup>	Sheep	20	Alfalfa hay + CF	Mix B	RBD	DMI, ADG, FCR
Canbolat and Karabulut <sup>22</sup>	Sheep	48	TMR	Oregano	FD	ADG
Chaves <i>et al.</i> <sup>7</sup>	Sheep	20	TMR	Juniper	RBD	DMI, ADG, FCR

Table 3. Main characteristics of the studies included in the meta-analysis.

n: number of experimental animals; CF: concentrate feed; TMR: total mixed ration; 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; CRD: completely randomized design; RBD: randomized block design; FD: factorial design; DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.

(DMI)				Effect Size	•	
Study name	Hedges' g (95% Cl)	-4.00	-2.00	0.00	2.00	4.00
Aydin <i>et al</i> . <sup>23</sup>	-0.32 (-1.35 to 0.68)		F			
Ribeiro <i>et al</i> . <sup>25</sup>	-0.04 (-0.77 to 0.68)			<b>—</b>		
Parvar <i>et al</i> . <sup>26</sup>	-0.55 (-1.30 to 0.17)		F	<b></b>		
Canbolat <i>et al</i> . <sup>11</sup>	-0.01 (-0.74 to 0.71)			<b>—</b>		
Malekkhahi <i>et al</i> . <sup>9</sup>	-0.38 (-1.76 to 0.91)				I	
Özdoğan <i>et al</i> . <sup>28</sup>	0.13 (-0.76 to 1.04)				H	
Chaves <i>et al</i> . <sup>7</sup>	0.24 (-0.65 to 1.16)			- <b>-</b>	-	
Overall	-0.12 (-0.50 to 0.26)			H		
(ADG)				Effect Size	•	
Study name	Hedges' g (95% Cl)	-4.00	-2.00	0.00	2.00	4.00
Aydin <i>et al</i> . <sup>23</sup>	0.23 (-0.77 to 1.26)			<b></b>		
Ribeiro <i>et al</i> . <sup>25</sup>	0.23 (-0.49 to 0.96)			<b>—</b> —	4	
Lei <i>et al</i> . <sup>8</sup>	1.19 (0.53 to 1.88)				<b>—</b>	
Parvar <i>et al</i> . <sup>26</sup>	-0.19 (-0.92 to 0.53)			<b>—</b>		
Yesilbag <i>et al</i> . <sup>27</sup>	0.33 (-0.43 to 1.11)			- <b>-</b>	-	
Gümüş <i>et al</i> . <sup>22</sup>	0.32 (-0.92 to 1.61)					
Özdoğan <i>et al</i> . <sup>28</sup>	0.68 (-0.22 to 1.64)			·		
Canbolat and Karubulut <sup>21</sup>	0.10 (-1.68 to 1.94)					
Chaves <i>et al</i> . <sup>7</sup>	0.71 (-0.19 to 1.68)			·	—	
Overall	0.44 (0.12 to 0.76)			H		
(FCR)				Effect Size	•	
Study name	Hedges' g (95% Cl)	-4.00	-2.00	0.00	2.00	4.00
Aydin <i>et al</i> . <sup>23</sup>	-0.20 (-1.22 to 0.81)		F			
Parvar <i>et al</i> . <sup>26</sup>	-0.17 (-0.90 to 0.55)					
Canbolat <i>et al</i> . <sup>11</sup>	0.36 (-0.36 to 1.10)				-	
Baytok <i>et al</i> . <sup>24</sup>	-0.15 (-0.93 to 0.62)			<b>—</b>		
Malekkhahi <i>et al</i> . <sup>9</sup>	-0.02 (-1.34 to 1.30)		F			
Özdoğan <i>et al</i> . <sup>28</sup>	-0.60 (-1.55 to 0.29)		⊢			
Chaves et al. <sup>7</sup>	-0.61 (-1.55 to 0.29)		<b>⊢</b>			
Overall	-0.17 (-0.55 to 0.22)			H		

Figure 2. 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.

# 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	<b>Missing studies</b>
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.

on DMI and FCR. 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 metaanalysis'. https://doi.org/10.6084/m9.figshare.12298913.v3<sup>30</sup>. 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<sup>13</sup>.

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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# **Open Peer Review**

# Current Peer Review Status:

Version 1

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# Liang Chou Hsia 🔟

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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?  $\ensuremath{\mathsf{Yes}}$ 

Are sufficient details of the methods and analysis provided to allow replication by others?  $\ensuremath{\mathsf{Yes}}$ 

Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review?  $\ensuremath{\mathsf{Yes}}$ 

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.

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.

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?  $\ensuremath{\mathsf{Yes}}$ 

# Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review?  $\ensuremath{\mathsf{Yes}}$ 

*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 Metaanalysis. 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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