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Research article

Evaluation of essential oil supplementation as a feed additive on rumen fermentation characteristics and methane mitigation in ruminants: A meta-analysis

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Abstract

This research aimed to investigate the use of essential oils (EO) in vivo as a feed additive on rumen fermentation characteristics and methane gas through a meta-analysis approach by integrating various related studies. The data analyzed came from 21 journals, 26 studies, and 73 data points from 2007–2021, which were tabulated using Microsoft Excel and analyzed with SAS using the mixed model method. The results of EO supplementation exhibited a significant ($P < 0.01$) decrease in ammonia concentration, Diplodinium population, and methane gas production, as well as a significant ($P < 0.05$) reduction of acetate proportion and Dasytrica populations. In contrast, EO supplementation had a significant increase in dry matter digestibility ($P < 0.01$) and final body weight ($P = 0.05$). The crude protein digestibility tended to increase ($P < 0.1$) with EO supplementation. In conclusion, EO supplementation as a feed additive can positively affect rumen fermentability and methane gas production.

Keywords: Essential oil, Fermentability, In vivo, Methane, Ruminant

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INTRODUCTION

The increase in ruminant population indirectly contributes to the increase in greenhouse gases. According to Beauchemin et al. (2020), the digestive system of ruminants contributes substantially to the emission of enteric methane into the atmosphere, thereby influencing greenhouse gas emissions. Moss et al. (2000) explained about 71% production of methane comes from anthropogenic activities, two-thirds from agricultural activities and one-third from enteric fermentation. Enteric methane (CH4) emissions produced through the fermentation of feed in rumen and lower digestive tract by methanogenic archaea are released into the atmosphere through the mouth and, to a smaller extent, through the nostrils and anus.

The energy efficiency of feed decreases with increasing methane gas production in ruminants (Jayanegara, 2009). Methane gas production is related to a loss of feed energy of 2-12% (Goel dan Makkar, 2012). Optimization of rumen function is the key to increase feed nutrient efficiency. Durmic et al. (2014) explained that feed additives could improve feed quality, break down anti-nutritional factors, and reduce methane production through manipulation of the rumen. However, feed additives such as antibiotics have been banned in the European Union and Indonesia since January 2006 and 2018, respectively due to the potential for antibiotic-resistant bacterial strains to leave residues in livestock products (Tajodini et al., 2014). Therefore, many studies have focused on alternative antibiotics using natural additives that contain high levels of secondary metabolites.

Essential oils (EO) are bioactive compounds derived from plants that have antimicrobial properties against various microorganism, such as bacteria, viruses, and fungi (Bakkali and Idaomar, 2008). The main components of EO are terpenoids and phenolic compounds (Benchaar and Greathead, 2011). Various studies have demonstrated that EO reduce methane gas production in ruminants. However, the research results continue to vary. According to Patra (2011), EO have a positive effect on carbohydrate and protein degradation, ammonia production, volatile fatty acids, and production of methane. Nonetheless, Benchaar et al. (2021) explained that EO had no significant effect on fermentation of rumen. Therefore, this study aims to investigate the impact of EO as a feed additive on in vivo rumen fermentation characteristics and production of methane through a meta-analysis by integrating data from multiple related studies.

MATERIALS AND METHODS

Database development

The database was developed from published research publications which record EO supplementation as a feed additive for ruminants *in vivo.* Article search via Scopus, Google Scholar, PubMed, and ScienceDirect with the keywords “essential oil,” and “ruminant”. The study developed by the database focuses on four types of essential oils that are commonly used in *in vivo* studies, including garlic, cinnamon, oregano, and thyme. The other inclusion criteria for articles in the database consisted of articles were that: (1) involved *in vivo* ruminant research; (2) the independent variable in one treatment should be EO, and (3) the studies have been published in the English language. The database incorporated many factors, such as rumen pH, ammonia (NH_3), total VFA, acetate (C_2), propionate (C_3), butyrate (C_4), iso-butyrate (iso-C_4), valerate (C_5), iso-valerate (iso-C_5), acetate-to-propionate ratio (C_2:C_3), total bacteria, total protozoa, *Dasytrica* population, *Diplodinium* population, methane gas (CH_4), dry matter digestibility (DMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), acid detergent fiber digestibility (ADFD), dry matter intake (DMI), initial body weight (IBW), final body weight (FBW), average daily gain (ADG), and feed conversion ratio (FCR). An examination of outliers was conducted utilizing the z-score method to confirm data points that significantly varied from the overall distribution of data for specific parameters. The final database consisted of 21 studies with 73 data points. Methane emissions were estimated using the stoichiometric formula from the partial VFA profile reported by *Moss et al.* (2000). A comprehensive overview of the studies that have been incorporated into the meta-analysis is shown in Table 1. The level of EO in this research varied from 0 to 2500 mg/kg, with a mean value of 310 mg/kg DM. The outcomes analysis of descriptive statistics is presented in Table 2.

Table 1 Studies utilized for meta-analysis of essential oils as a feed additive in ruminant *in vivo*

No	References	EO	Ratio C: F	Animal	Level EO (mg/kg)
1	Yang et al. (2007)	Garlic	60:40	Cow	0-5000
2	Chaves et al. (2008)	Cinnamon, garlic	80:20	Sheep	0-200
3	Chaves et al. (2008)	Oregano, cinnamon	100:0	Sheep	0-200
4	Benchaar et al. (2008)	Cinnamon	60:40	Sheep	0-1000
5	Yang et al. (2010)	Cinnamon	85:15	Cow	0-1600
6	Koyuncu et al. (2010)	Oregano	TMR	Cow	0-450
7	Yang et al. (2010)	Cinnamon	85:15	Cow	0-1600
8	Chaves et al. (2011)	Cinnamon	70:30	Sheep	0-400
9	Anassori et al. (2011)	Garlic	12:78	Sheep	0-750
10	Zijderveld et al. (2011)	Garlic	66:34	Cow	0-200
11	Vakili et al. (2013)	Thyme, cinnamon	15:85	Cow	0-5000
12	Biricik et al. (2016)	Oregano, thyme	100:0	Sheep	100-300
13	Tefaghi et al. (2017)	Thyme	100:0	Cow	0-598
14	Kolling et al. (2018)	Oregano	60:40	Cow	0-560
15	Benchaar (2019)	Oregano	60:40	Cow	0-50
16	Benchaar (2020)	Thyme	50:50	Cow	0-50
17	Ozkaya et al. (2020)	Oregano	100:0	Cow	0-200
18	Ribeiro et al. (2020)	Thyme	10:90	Sheep	25-2500
19	Canbolat et al. (2021)	Garlic	100:0	Sheep	0-1200
20	Tawab et al. (2021)	Thyme	40:60	Sheep	0-520
21	Zhou et al. (2021)	Oregano	65:35	Sheep	0-7000

Note: EO= essential oil; C= concentrate; F= forage

Table 2 Descriptive statistics of effects EO supplementation on rumen fermentation profile, feed digestibility, and performance productivity *in vivo*

Variable	Unit	n	Minimum	Maximum	Mean	SD
EO level	mg/kg	73	0.00	2500	310.68	52.46
Fermentation Profile						
pH		66	5.39	6.70	6.04	0.33
NH_3	mM	56	3.08	20.35	10.28	4.24
Total VFA	mM	66	81.00	144.20	112.33	18.72
C_2	%	64	47.00	66.36	57.56	5.98
C_3	%	68	18.70	43.60	28.97	7.66
C_4	%	66	5.68	17.50	11.78	2.9
Iso-C_4	%	10	0.68	1.02	0.93	0.1
C_5	%	34	1.28	3.00	1.76	0.48
IsoC_5	%	11	0.49	2.44	1.48	0.59
BCVFA	%	19	2.40	7.20	3.99	1.67
Ratio C_2:C_3		69	0.85	3.41	2.14	0.77
Total Bacteria	log cfu/ml	7	9.11	10.69	9.8	0.8
Total Protozoa	log cell/ml	23	3.73	6.62	5.27	0.99
Diplodinium	log cell/ml	11	2.52	5.01	3.49	1.16
Dasytrica	log cell/ml	6	4.07	5.78	5.13	0.7
Methane gas	mM	68	10.42	30.14	22.70	5.26
Feed digestibility						
DMD	%	16	53.00	85.33	69.13	8.69
CPD	%	24	55.30	90.03	77.80	12.42
NDFD	%	30	28.30	76.75	56.82	11.63
ADFD	%	16	42.10	68.90	52.25	5.99
Performance productivity						
DMI	kg/d	31	1.02	1.93	1.33	0.23
IBW	kg	28	19.50	39.50	23.89	4.36
FBW	kg	24	38.70	53.30	43.78	3.31
ADG	kg/d	28	0.11	0.37	0.27	0.06
FCR		26	4.03	5.30	4.57	0.37

Note: NH3= ammonia; VFA= volatile fatty acid; C2= Acetate; C3= propionate; C4= butyrate; C5= valerate; BCVFA= branched chain volatile fatty acids; DMD= dry matter digestibility; CPD= crude protein digestibility; NDF= neutral detergent fiber; ADF= acid detergent fiber; DMI= dry matter intake; IBW= initial body weight; FBW= final body weight; ADG= average daily gain; FCR= feed conversion ratio.

Data Statistical Analysis

The current research applied a mixed model meta-analysis methodology (St-Pierra, 2001; Sauvant et al., 2008) to analyze the data collected with statistics. The data analysis was conducted with the PROC MIX method within the SAS program. The study incorporated a random effect, with the EO level treated as a fixed effect. The predictor variable that was continuously measured in this study was the level of EO supplementation as follow:

where e_{ij}= dependent variable; e_{ij}= overall intercept of studies (fixed effect); e_{ij}= coefficient linear regression of Y on X (fixed effect); e_{ij}= coefficient quadratic regression of Y on X (fixed effect); e_{ij}= value of the continuous predictor variable (level of EO); e_{ij}= value of random effect of study i; e_{ij}= random effect of study on the regression coefficient of Y on X in study i; and e_{ij}= residual error. The model is considered very significant at $P < 0.01$ or has a significant trend between $0.05 < P < 0.10$

RESULTS

Fermentation profile

EO supplementation showed a significant decrease ($P < 0.01$) in ammonia concentrations and Diplodinium populations. Additionally, the proportion of acetate, Dasytrica populations, and methane gas was a significant decrease ($P < 0.05$). However, EO supplementation had no effect of the other fermentation profile such as pH, total VFA, propionate, butyrate, iso-butyrate, valerate, iso-valerate, branched chain volatile fatty acids, acetate-propionate ratio, total bacteria and protozoa population. Results of statistical analysis of the response of EO supplementation to rumen fermentation parameters shown in Table 3.

Table 3 Results of statistical analysis of the response of EO supplementation to rumen fermentation parameters

Variable	Unit	n	Model	Parameter Estimate				Model Estimate
				Int	SE Int	Slope	SE Slope	p-value
pH		66	L	6.07	0.07	0.002	0.008	0.80
NH_3 (mM)	mM	56	L	10.95	1.02	-0.002	0.006	<0.01
Total VFA	mM	66	L	112.8	4.05	-0.003	0.003	0.43
C_2	%	64	L	58.75	1.32	-0.002	0.001	0.04
C_3	%	68	L	28.69	1.70	-0.004	0.009	0.66
C_4	%	66	L	11.36	0.66	-0.006	0.004	0.18
Iso-C_4	%	10	L	0.92	0.06	-0.002	0.001	0.87
C_5	%	34	L	1.82	0.16	-0.002	0.009	0.85
Iso-C_5	%	11	L	1.47	0.37	-0.002	0.001	0.24
BCVFA	%	19	L	4.22	0.78	-0.001	0.003	0.70
Ratio C_2:C_3		69	L	2.26	0.16	-0.001	0.007	0.20
Bacteria	log cfu/ml	7	L	9.92	0.74	-0.002	0.003	0.56
Protozoa	log cell/ml	23	L	5.41	0.35	0.008	0.002	0.76
Diplodinium	log cell/ml	11	L	3.56	0.72	-0.003	0.004	<0.01
Dasytrica	log cell/ml	6	L	5.05	0.69	-0.002	0.005	0.03
Methane gas	mM	68	L	23.21	1.15	-0.013	0.005	0.01

Note: NH_3= ammonia; VFA= volatile fatty acid; C_2= acetate; C_3= propionate; C_4= butyrate; C_5= valerate; BCVFA= branched chain volatile fatty acids; L= linear

Feed Digestibility and Performance Productivity of Ruminant

In Table 4, essential oils supplementation is shown to have significant increase in DMD (P < 0.01) and FBW (P = 0.05), and showed a tendency to increase CPD (P < 0.1). On the other hand, essential oil had no significant effects on NDFD, ADFD, DMI, IBW, ADG, and FCR.

Table 4 Results of statistical analysis of the response of EO supplementation to feed digestibility and performance productivity

Variable	Unit	n	Model	Parameter Estimate				Model Estimate
				Int	SE Int	Slope	SE Slope	p-value
Feed Digestibility								
DMD	%	16	L	66.39	3.61	0.02	0.005	<0.01
CPD	%	24	L	75.18	4.77	0.002	0.001	0.08
NDFD	%	30	L	57.91	3.59	-0.003	0.002	0.15
ADFD	%	16	L	52.48	2.50	-0.001	0.004	0.77
Performance Productivity								
DMI	kg/d	31	L	1.37	0.08	<-0.01	0.003	0.70
IBW	kg	28	L	29.1	4.66	0.005	0.005	0.36
FBW	kg	24	Q	43.9	1.30	0.010	0.003	0.05
						<-0.01	<-0.01	0.03
ADG	kg/d	28	L	0.26	0.02	<-0.01	<0.01	0.11
FCR		26	L	3.99	0.56	<0.001	<0.001	0.52

Note: DMD= dry matter digestibility; CPD= crude protein digestibility; NDF= neutral detergent fiber; ADF= acid detergent fiber; DMI= dry matter intake; IBW= initial body weight; FBW= final body weight; ADG= average daily gain; FCR= feed conversion ratio; L= linear; Q= quadratic.

DISCUSSION

The supplementation of EO as a feed additive on the rumen fermentation did not have a significant effect on the pH levels in the rumen. In line with the addition of cinnamon and garlic EO at a dose of 200 mg/d and oregano (100 mg/d) and thyme (300 mg/d) in sheep (Bİrİcİk et al., 2016). Furthermore, the findings of Kolling et al. (2017) and Benchaar (2019) regarding dairy cows indicate that the use of oregano essential oil also did not provide a significant effect on rumen pH level. The insignificant effect of rumen pH proves that the antimicrobials contained in EO can maintain pH so that it does not interfere with the rumen fermentation process. According to Patra (2011), the antimicrobial activity of EO can improve feed quality without interfering with rumen fermentation.

The ammonia concentration decreased significantly (P < 0.01) after EO supplementation. This result aligns with Newbold et al. (2004) in sheep with EO supplementation of 0.11 g/day. The decrease in ammonia concentration was due to the ability of essential oils to modify protein metabolism and antimicrobial activity in rumen (Kamalak et al., 2011; Besharati et al., 2021). McIntosh et al. (2003), reported that the antimicrobial activity of EOs in inhibiting excess hyper-ammonia-producing (HAP) which are classified as phenolic compounds can protect feed protein from degradation. This occurrence results in a decrease in the availability of nutrients to cells, leading to the suppression of bacterial proliferation. According to Macheboeuf et al. (2008) and Cobellis et al. (2015), a decrease in rumen ammonia concentration implies an increase in the availability of amino acids that can be absorbed in the small intestine.

The meta-analysis of EO supplementation did not show a significant effect on total VFA. Benchaar et al. (2007) reported similar results for cinnamaldehyde EO in dairy cow rations. According to previous studies conducted by Martinez et al. (2006); Benchaar et al. (2007); and Khorrami et al. (2015), it was found that thyme essential oil did not have a significant impact on the overall levels of volatile fatty acids (VFAs) in both dairy cattle and beef cattle. In a recent investigation carried out by Canbolat (2021), it was shown that the application of garlic-derived essential oil resulted in a significant decrease in the overall concentration of volatile fatty acids (VFAs) in sheep, specifically at a dosage of 1200 mg/d. Foskolos (2015), explained that supplementation with lower amounts of EO has little or no effect on total VFA, and higher doses can interfere with overall microbial activity. Furthermore, it was observed that there was significant decrease the proportion of acetate. These results align with research conducted by Canbolat et al. (2021), who reported a decrease in acetate concentration after feed supplemented with essential oil from garlic. However, EO supplementation had no significant effect on the proportions of propionate, butyrate, valerate, and BCVFA. Decreasing acetate concentration is suspected to inhibit acetate-producing bacteria. Generally, acetate is produced by gram-negative bacteria such as *F. succinogenes, R. flavefaciens,* and *R. albus* (Wallace et al., 2002). The *F. succinogenes* population suffered from more inhibition than the populations of *R. flavefaciens* and *R. albus* for all EOs (Patra and Yu, 2012). The mechanism of thymol essential oil in inhibiting gram-negative bacteria, according to Zhou et al. (2007), includes destroying the outer membrane of gram-negative bacteria, releasing lipopolysaccharide, increasing the permeability of the cytoplasmic membrane, thereby allowing ions to leave the cell cytoplasm. Meanwhile, cinnamaldehyde can cause cell membrane shrinkage and invagination in acetate-producing bacteria, which causes cell membrane damage and disrupts intracellular homeostasis (Wang et al., 2022).

The proportion of BCVFA had no significant effect. This is consistent with the research of Benchaar (2021), which included the addition of oregano to dairy cattle ration. This suggests that the antimicrobial EOs did not inhibit the activity of iso-butyrate and iso-valerate-producing bacteria and the accumulation of both, specifically BCVFA (Zhang et al., 2021). In this study, the insignificant effect on the acetate-to-propionate ratio was consistent with the significant decrease in the proportion of acetate, whereas propionate had no significant effect.

The supplementation of EO did not have a significant impact on the overall population of bacteria and protozoa. This finding align with the results reported by Benchaar et al. (2007), which indicated that there was no significant alteration in the overall population of bacteria and protozoa. However, the *Diplodinium* population decreased significantly ($P < 0.01$) and the *Dasytrica* population decreased significantly ($P < 0.05$). Essential oils are thought to disrupt the integrity of cell membranes in protozoa. According to Cox et al. (2001), the ability of essential oils as antimicrobials can increase potassium ion leakage, thereby disrupting cell membrane permeability.

Furthermore, ciliated protozoa are involved in the process of breaking down high-fiber diet. The decline in the population of ciliated protozoa is consistent with the findings of Soroor and Rouzbehan (2017). According to Dai and Faciola (2019) ciliated protozoa have a symbiosis with ruminal methanogens. This is due to the activity of protozoa in degrading fibers to produce hydrogen gas, and methanogens can use the hydrogen to form methane. Therefore, the decrease in ciliated protozoa indirectly positively impacts methane gas production.

The addition of the EO significantly decreased methane gas ($P < 0.01$). Kolling et al. (2018) also reported similar results. The reduction in methane gas production in this study may be attributed to the strong antibacterial abilities of essential oils, which have the ability to influence the microbial population responsible for methane gas generation. This is corroborated by a decline in acetate production and ciliated protozoa, such as *Diplodinium* and *Dasytricha*. According to Soroor and Rouzbehan (2017), up to 34% of the methanogenesis in the rumen is caused by ciliated protozoa. The ability of EO to reduce acetate production and inhibit the proliferation of ciliated protozoa is positively correlated with a decrease in methane gas production. This is because less hydrogen is available to methanogens to produce methane gas.

EO supplementation significantly increased DMD ($P < 0.01$) and showed a tendency to increase CPD ($P < 0.1$). However, NDFD and ADFD did not have a significant effect. The observed increase in DMD is consistent with the findings of Zhou et al. (2020) regarding the effects of oregano essential oil intake. The study conducted by Abd El Tawab et al. (2020) demonstrated a direct correlation between the rise in DMD and CPD. The lengthening of rumen papillae can lead to an increase in DMD and CPD. The alteration in papillae length could enhance the surface area for nutrient contact, thereby aiding in the thorough breakdown of feed and subsequently improving digestion and feed efficiency (Liu et al., 2020). In accordance with the findings of Zhang et al. (2021), the addition of oregano EO lengthened the rumen papilla. This correlates positively with increased feed digestibility in the rumen. The trend of increasing CPD was inversely proportional to the significant decrease in ammonia concentration. According to Kholif et al. (2018) EO has a post-ruminal effect, which decreases the concentration of ammonia in the rumen and increases CPD In addition, Abd El Tawab et al. (2020) explained that thyme EO could inhibit feed protein degradation, thereby increasing the digestible protein supply in the lower intestine of ruminants. This shows that essential oils can increase protein utilization (Besharati et al., 2021). The lack of effect on the digestibility of NDFD and ADFD is thought to be due to the antimicrobial properties of the essential oils not inhibiting the work of all fiber digesting microorganisms (Yang et al., 2010). Besides that, Yang et al. (2009) reported the essential oils mechanism seems to be mainly in the rumen, thus affecting rumen digestion with minimal effect on intestinal digestion.

EO supplementation had no significant effect on the DMI, IBW, ADG, and FCR of small ruminants. According to the findings of Bampidis et al. (2005), there was no significant change observed in the feed consumption and feed conversion rates of sheep that were supplemented with oregano. This observation aligns with Benchaar et al. (2007) and Yang et al. (2010). Burt (2004) argues that essential oils exhibit distinct flavors and fragrances that vary depending on their origin. Consequently, this will have an effect on the daily intake of animal feed that is enhanced with essential oils. Nevertheless, EO supplementation shown a significant effect ($P < 0.05$) on increasing FBW. The findings of this study are consistent with Akbarian-Tefaghi et al. (2018).

The increase in BW aligns with the increase in feed digestibility in the rumen. According to Zhang et al. (2021), the study outcomes indicate that essential oils have the potential to stimulate elongation of intestinal papillae, leading to enhanced digestibility of animal feed. Consequently, it may have an indirect effect on promoting an increase in body weight. In addition, Hang et al. (2021) found that the inclusion of oregano essential oil as a feed additive had a positive effect on livestock performance.

CONCLUSIONS

It was concluded that EO supplementation could protect feed proteins and inhibit the ammonia production. The EO can then reduce the proportion of acetate and the population of ciliated protozoa such that it is positively correlated with a decrease in methane gas production without affecting livestock performance.

AUTHOR CONTRIBUTIONS

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AUTHOR CONTRIBUTIONS

Dessy Permata: Conceptualization, Methodology, Investigation, Data curation, Validation, Visualization, Writing – original draft.
Komang G. Wiryawan: Validation, Writing – review & editing,
Anuraga Jayanegara: Methodology, Validation, Writing – review & editing.

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