Veterinary Integrative Sciences 2023; 21(3): 925 - 944 DOI; 10.12982/VIS.2023.067

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Effects of essential oils and their derivatives on rumen fermentation characteristics and PUFA biohydrogenation: A meta-analysis of *in vitro* studies

Malik Makmur[1,7,*], Yulianri Rizki Yanza[3,4,7], Ainissya Fitri[2,7], Syarifuddin[5], Roni Ridwan[2,7] and Anuraga Jayanegara[6,7]

[1]National Research and Innovation Agency (BRIN), Cibinong 16911, West Java, Indonesia.
[2]Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong 16911, West Java, Indonesia.
[3]Department of Biology Education, Faculty of Teacher Training and Education, Riau Islamic University (UIR), Pekanbaru 28284, Riau, Indonesia
[4]Department of Animal Feed Nutrition and Technology, Faculty of Animal Science, Padjadjaran University, Jatinangor, 45363, West Java Indonesia
[5]Animal Husbandry Study Program, Agriculture Faculty, Bosowa University, Makassar 90231, Indonesia
[6]fDepartment of Nutrition and Feed Technology, IPB University, Bogor 16680, West Java, Indonesia
[7]Animal Feed and Nutrition Modelling (AFENUE) Research Group, IPB University, Bogor 16680, West Java, Indonesia

Abstract

The present meta-analysis study was aimed to determine effects of essential oil and their derivatives (EOD) supplementation as natural additives on rumen fermentation characteristics and rumen biohydrogenation (BH) activity *in vitro*. A meta-analysis database was built from the 24 verified scientific articles and further all data were analysed through the continuous random effects model using OpenMEE. It was evaluated that the EOD levels up to 500 mg/L. It showed that high EOD levels increased pH and acetate concentration ($P < 0.001$) but reduced ammonia, propionate, the acetate to propionate ratio, total volatile fatty acid, and rumen gas production *in vitro* ($P < 0.001$). On the perspective of rumen fatty acids profile, the increased EOD levels also positively accumulating n-9 monounsaturated fatty acid, conjugated linoleic acid c9 t11, as well as n-6 and n-3 polyunsaturated fatty acid (PUFA) in the rumen after *in vitro* fermentation ($P < 0.001$), and consequently inhibited rumen biohydrogenation (BH) of n-6 and n-3 PUFA ($P < 0.001$). Meta-regression analysis also showed a negative correlation between EOD supplementation levels and the BH of C18:2 n-6, C18:3 n-3, C20:5 n-3, and C22:6 n-3. It was suggested that the EOD supplementation at 300 mg/L indicated a positive effect on modulating the rumen PUFA metabolism. Above all evidences, it can be concluded that EOD treatment can reduce rumen fermentability but increase the proportion of beneficial fatty acids and inhibit the rate of BH PUFA.

Keywords: Biohydrogenation, Essential oil, *In vitro*, Meta-analysis, Rumen

Corresponding author: Malik Makmur, National Research and Innovation Agency (BRIN), Cibinong 16911, West Java, Indonesia. Email: malikmakmur27@gmail.com; mali008@brin.go.id.

Article history; ***received manuscript: 18 June 2023,***
revised manuscript: 11 July 2023,
accepted manuscript: 13 July 2023,
published online: 21 July 2023
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Natural feed additives from phytogenic substances have shown beneficial effects on ruminants in several years, especially on rumen fermentation and production performance. Essential oils (EO) and their derivatives (EOD) are extracted phytogenic substance derived from plants containing secondary metabolite that can modulates rumen microbial due to their antioxidant or antibacterial activity during fermentation in the rumen (Calsamiglia et al., 2007; Nanon et al., 2014). Hence, volatile fatty acids (VFA) production and macronutrient metabolism in the rumen might also altered by the EOD mode of action. Moreover, previous studies revealed that essential oils not only alter rumen fermentation but also hampered ruminal biohydrogenation (BH) process of n-3 and n-6 fatty acids (FA) in the rumen, which are generally known that those FA groups are essentials for ruminant (Zhu et al., 2012; Gunal et al., 2013; Gunal et al., 2014). EOD supplementation may become importance due to the substance efficacy to inhibit the ruminal BH process, increase the PUFA absorption in the digestive tracks, and consequently improve the PUFA deposition in the certain ruminant tissues preserved as food products from ruminant origins, such as meat and milk.

Previous studies revealed that the essential oils together with bioactive components derivatives such as thymol, eugenol, cinnamaldehyde, and carvacrol supplementation in dairy cow rations were able to reduce the population of protozoa, methanogens, proteolytic bacteria, and bacteria involved in BH (Daning et al., 2020). However, supplementation of EOD at different levels with a diverse dietary source showed inconsistent results, especially when they are associated with rumen metabolism (Benchaar et al., 2008; Patra, 2011). Gunal et al. (2014) reported that the addition of up to 500 mg/L by several types of EO reduces rumen C18:0 and trans C18:1 proportion in the rumen *in vitro*. On the other hand, Eburu and Anya (2020) reported that the effect of anise EO at 450 mg/L level increased rumen C18:0 composition. A similar result was found in rumen VFA production from recent studies, where garlic, cinnamon, oregano EO, and carvacrol supplementation had shown a non-significant change (Doreau et al., 2017; Benchaar, 2020). In contrast, Daning et al. (2022) reported a significant decrease in rumen VFA production with galangal EO treatments. Therefore, a meta-analysis approach is important to robust the conclusion regarding effects of the EOD supplementation on rumen fermentation characteristics and FA modulation. Hence, the present meta-analysis was aimed to evaluate the effects of EOD supplementation as additives on rumen fermentation, fatty acids profile, and rumen biohydrogenation process. We expected that the present *in vitro* meta-analysis study may represent the underlying effects of EOD under *in vivo* conditions.

MATERIALS AND METHODS

Database development

Integration of the study data on the effects of EOD supplementation on fermentability and fatty acid metabolism in simulated *in vitro* rumen was used as the database in this meta-analysis study. The literature search strategy included

the keywords "essential oil", "rumen", "biohydrogenation," and "PUFA". In addition, the search employed the Scopus, Cambridge Core, Google Scholar, and ScienceDirect platforms. The inclusion criteria for studies were that: (1) they used EOD supplementation or plants rich in EOD content; (2) EOD treatment is not mixed with other substances; (3) there was a control treatment; and (4) rumen simulation was performed using *in vitro* techniques. The study search and selection criteria are shown in Figure 1. Database development followed the assessment items of the PRISMA protocol (Liberati et al., 2009). A total of 24 studies from 2008 to 2021 were collected in line with the predefined criteria and included in the meta-analysis (Table 1). The collected literature sources comprised peer-reviewed journals, seminar proceedings, and a PhD thesis. In addition, 27 EO types (including blend oil), 42 EO bioactive components, three EO-rich plant extracts, and two EO source plant materials were evaluated. The *in vitro* rumen methods were batch culture incubation, glass bottle incubation, the Hohenheim gas test, the pressure transducer technique, and used of a continuous culture fermenter. The rumen inoculum was sourced from various groups of large and small ruminants. The dose range or EOD level in the study was 0-500 mg/L. The literature search was completed in November 2022.

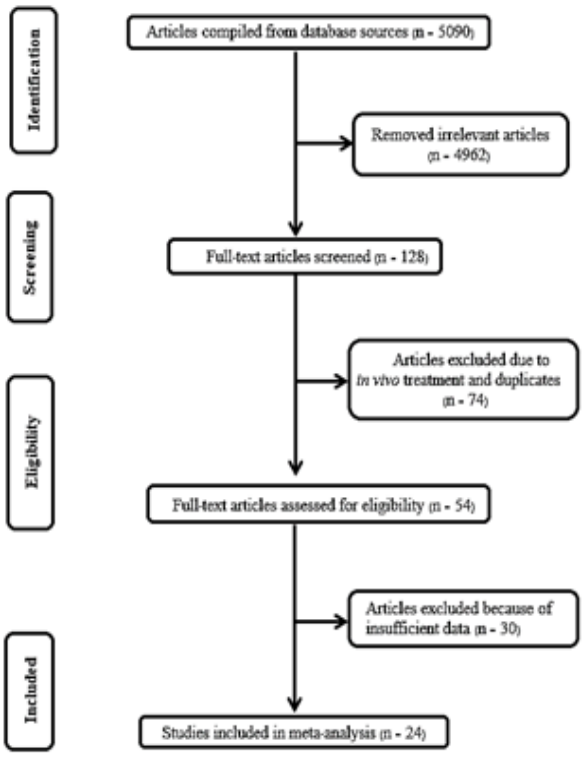

Figure 1 Flow chart of selected study.

Table 1 Studies included in meta-analysis database

No.	Study	*In vitro* methods	Incubation period (h)	Rumen inoculum	Lipid source	Treatment
1.	Bokharaeian and Chikunya (2012)	GBI	48	Unspecified	Mixture of fish oil and linseed oil	3-Carene, *p*-Cresol, Resorcinol
2.	Daning et al. (2021)	GBI	48	Dairy cattle	N/A	*Alpinia galangal* oil, Cineole
3.	Doreau et al. (2017)	BCI	5, 24	Sheep	Extruded linseed oil	Cinnamon oil, Garlic oil
4.	Eburu and Anya (2018)	PTT	24, 48	Goats (West African Dwarf)	Fish oil and whole ground wheat grain	*Pimpinella anisum* oil, *Lavandula angustifolia* oil, Blend oil (*P. anisum* and *L. angustifolia*)
5.	Eburu and Chikunya (2014)	GBI	48	Ewes (Hartline Texel cross)	Mixture of fish oil and linseed oil	Eucalyptol, 3-Carene, *trans*-Anethole, Borneole, 4-Allylanisole, *trans*-Cinnamaldehyde, β, Citronellol, Limonene, Menthol, Myrtenol, *P*-Cymene, α-Thujone
6.	Eburu and Chikunya (2015a)	GBI	48	Ewes (Hartline Texel cross)	Mixture of fish oil and linseed oil	Anise oil, Cassia oil, Citronella oil, Clove oil, Cornmint oil, Eucalyptus oil, Juniper berry oil, Lavender oil, Mandarin oil, Rosemary oil
7.	Eburu and Chikunya (2015b)	GBI	24	Ewes (Hartline Texel cross)	Mixture of fish oil and linseed oil	4-Allylaniasole, Anethole, Anise oil, Cassia oil
8.	Eburu et al. (2017)	PTT	12, 24, 48	Lambs (Hartline Texel cross)	Mixture of fish oil and linseed oil	*Pimpinella anisum* oil
9.	Eburu and Anya (2020)	PTT	24	Goats (West African Dwarf)	Fish oil and whole ground wheat grain	*Pimpinella anisum* oil
10.	Foskolos et al. (2015)	CCF	24	Cow (Holstein)	N/A	Propyl-propane thiosulfonate
11.	Gilman and Chikunya (2014)	PTT	24	Unspecified	Mixture of fish oil and linseed oil	Clove oil, Eugenol, Eugenyl acetate, *trans*-Caryophyllene
12	Guerreiro et al. (2016)	HT	6	Lambs (Merino Baranco	Sunflower oil	*Cistus ladanifer* oil
13.	Gunal et al. (2013)	BCI	24	Cows (Holstein)	Soybean oil	*Abies sibirica* oil, *Cymbopogon winterianus* oil, *Rosmarinus officinialis* oil, *Salvia officinialis* oil, *Thymus vulgaris* oil, *Eugenia caryophyllus* oil
14.	Gunal et al. (2014)	BCI	24	Cows (Holstein)	Soybean oil	*Illicium verum* oil, *Juniperus virginiana* oil, *Cinnamomum cassia* oil, *Eucalyptus globulus* oil, *Melaleuca alternifolia* oil
15.	Ishlak et al. (2015)	CCF	24	Cows (Holstein)	Corn oil	Cinnamaldehyde
16.	Lourenço et al. (2008)	CCF	24	Cows (Holstein)	N/A	Quercetin, Eugenol, Cinnamaldehyde
17.	Lourenço et al. (2009)	GBI	24	Cows (Holstein)	Mixture of linseed oil and sunflower oil	Alpine vegetation oil
18.	Mandal et al. (2016)	GBI	24	Goats	Sunflower oil	*Syzygium aromaticum* buds extract, *Allium sativum* bulb extract, *Coriandum sativum* seed extract
19.	Ramos-Morales et al. (2013)	CCF	24	Goats (Murciano-Granadina)	Linoleic acid	Diallyl disulfide, Propyl-propane thiosulfinate
20.	Purba et al. (2020)	HGT	24	Goats (Saanen)	Sunflower oil	*Piper betle* leaf powder
21.	Sgwane (2014)	PTT	24	Ewes (Suffolk cross), Lambs (Texel)	Mixture of fish oil and ground linseed	*cis*-3-Hexen-1-ol, Geraniol, Linalool, Nerol, Terpinen-4-ol, α-Terpineol, *cis*/*trans*-Citral, (+)-Camphene, (+)-α-Pinene, Linalyl acetate, Geranyl acetate, l-Carvone, l-Fenchone, l-Menthone, (R)-(+)-Pulegone, (1S)-(-)-Verbenone, Carvacrol, Eugenol, Guaiacol, Thymol
22.	Siurana et al. (2018)	BCI, CCF	6, 24	Dairy cows	Linseed oil	Oxy-propyl-thiosulfate, Eugenol, Cinnamaldehyde
23.	Tiven (2017)	HGT	48	Sheep	Crude palm oil	*Citrus hystrix* leaf
24.	Ye et al. (2018)	CCF	24	Cows (Holstein)	Corn oil	Blend oil (cinnamaldehyde and garlic)

Note: BCI = batch culture incubation; GBI = glass bottle incubation; HT = hungate tubes; HGT = Hohenheim gas test; PTT = pressure transducer technique; CCF = continuous culture fermenter; N/A = not available

Data analysis

Data integrated into the meta-analysis database in terms of sample size, mean value, and standard deviation or standard error of measurement in the control and EOD treatments were converted into Hedges' d effect size values (Gurevitch and Nakagawa, 2015). Previously, descriptive analysis (Table 2) was conducted on all the tabulated variable data collected. The Spearman rank correlation (r) was also conducted on the data related to fermentation characteristics with rumen BH fatty acid products to determine the correlation strength between the variables. The correlation pattern between the data was then interpreted into a correlogram (Schoonjans, 2017). Subsequently, the variables of fermentability (14 items), fatty acid composition (16 items), and rumen BH PUFA (4 items) were analysed through a continuous random effects approach (95% confidence interval or CI) with the following equation:

$$y_i = \theta + v_i + \varepsilon_i$$

where y_i is the Hedge's d value as an effect size; θ is the estimate value of the selected publication; v_i is the effect size value heterogeneity; and ε_i the error estimate of selected publication. Calculation of Hedges' d effect size, between-study heterogeneity (I^2), and meta-analysis was made using OpenMEE software (http://www.cebm.brown.edu/openmee/). The detection of publication bias was based on selected models for random effects and weight function plots (Bartoš et al., 2022). Furthermore, the determination and visualisation of publication bias plots were run on JASP software (http://jasp-stats.org/). Correlations between the effect size (ES) of BH rumen PUFA and EOD levels are shown in equations and meta-regression plots (95% CI and 95% prediction). MedCalc (http://www.medcalc.org/) was used to create correlograms and meta-regression analyses.

Table 2 Descriptive statistics of meta-analysis database

Variable	Unit	n	Mean	Minimum	Maximum	SD
Rumen degradability rates						
IVDMD	%	14	48.26	44.73	62.00	0.42
IVOMD	%	8	56.24	38.20	66.40	2.99
Fermentation profile						
pH		75	6.73	6.36	7.07	0.09
Ammonia	mg/dL	97	17.02	3.00	44.82	1.83
Acetate	mol/100 mol	98	53.71	2.70	95.80	1.60
Propionate	mol/100 mol	98	21.38	1.80	33.50	1.06
Butyrate	mol/100 mol	86	15.67	1.00	24.94	1.08
iso-Butyrate	mol/100 mol	46	3.03	0.15	4.90	0.23
Valerate	mol/100 mol	53	4.93	0.20	7.26	0.50
iso-Valerate	mol/100 mol	46	5.59	0.15	8.09	0.37
A/P		90	2.60	1.12	9.21	0.20
Total VFA	mM	159	63.22	6.00	147.00	3.82
Gas production 24 h	ml/g OM	48	123.88	36.80	172.00	2.93
Gas production 48 h	ml/g OM	28	106.51	54.10	194.60	4.95
Fatty acid profile						
C14:0	g/100 g TFA	48	4.14	0.44	7.68	0.51
C16:0	g/100 g TFA	82	17.47	9.66	53.28	1.63
C16:1	g/100 g TFA	33	2.62	0.06	3.80	0.05
C18:0	g/100 g TFA	162	17.28	0.55	71.28	5.49
C18:1 n-9	g/100 g TFA	78	8.24	1.51	39.89	3.30
C18:1 t	g/100 g TFA	34	5.14	2.93	13.36	0.42
C18:1 t11	g/100 g TFA	184	5.06	0.83	21.65	1.63
C18:2 n-6	g/100 g TFA	192	5.70	0.97	30.52	3.23
CLA c9 t11	g/100 g TFA	199	0.32	0.02	10.90	0.27
CLA t10 c12	g/100 g TFA	59	0.14	0.01	0.77	0.38
C18:3 n-3	g/100 g TFA	191	5.55	0.11	31.40	0.95
C20:5 n-3	g/100 g TFA	118	2.27	0.3	4.43	0.07
C22:6 n-3	g/100 g TFA	118	1.98	0.70	2.91	0.05
SFA	g/100 g TFA	51	42.20	18.50	80.45	13.99
MUFA	g/100 g TFA	48	16.71	10.30	39.89	1.62
PUFA	g/100 g TFA	48	13.21	2.10	59.60	1.06
Biohydrogenation activity						
C18:2 n-6	%	100	53.04	22.50	89.00	3.86
C18:3 n-3	%	102	61.47	26.10	89.80	3.81
C20:5 n-3	%	78	49.14	9.40	85.60	3.61
C22:6 n-3	%	78	27.77	3.90	71.10	3.63

RESULTS

Correlation between fermentation characteristics and rumen fatty acid

The relationship between rumen fermentation parameters and fatty acids from EO treatment showed a weak to moderate Spearman's correlation (r) (Figure 2). However, different pH levels of rumen fluid affected the fatty acid profile of BH intermediates, with a strong positive correlation (r = 0.627) to the proportion of CLA c9 t11. In addition, the valeric acid item showed a strong negative correlation (r = -0.680) to C18:1 t11 but a weak negative (r = -0.255) to C18:1 t. The main product of rumen BH, C18:0 fatty acids, showed a strong negative correlation to several rumen fermentation variables such as *iso*-valerate (r = -0.534), valerate (r= -0.534) and IVOMD (r = -0.548).

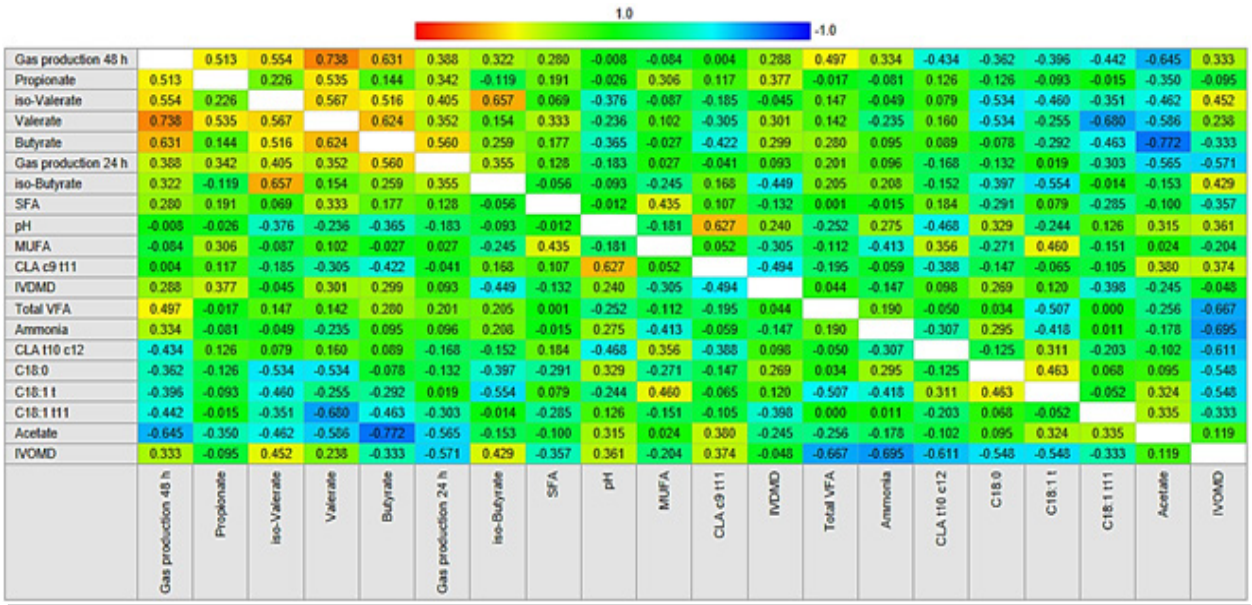

Figure 1 Correlogram of the relationship between *in vitro* rumen fermentation characteristics and rumen fatty acid treated with EOD.
Note: IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Fermentation profile

Descriptive statistics regarding the effect of the addition of EOD on rumen fermentability variables are showed in Table 2. The analysis showed a very significant increase in the addition of essential oils and their derivatives as ruminant feed additives to rumen fermentability ($P < 0.001$) on rumen pH and acetate concentration. However, it significantly decreased ($P < 0.001$) the production of ammonia, propionate, total VFA, and 24 h and 48 h gas production (Table 3). In addition, EOD treatment also significantly increased butyrate concentration ($P < 0.05$) and acetate: propionate ratio (A/P; $P < 0.001$) but lowered *iso*-valerate ($P < 0.01$). The variables of *iso*-butyrate, valerate, IVDMD, and IVOMD did not change significantly as the treatment dose increased. The test percentage I^2 on various rumen fermentation profile variables was in the range of 67.92 - 95.43%, which is in the high heterogeneity category.

Table 3 Effect of EOD supplementation on *in vitro* rumen fermentation characteristics

Variable	Unit	n	Estimate	Lower bound	Upper bound	Std. error	P-Value	τ^2	Q	Het. P-value	I^2
Rumen degradability rates											
IVDMD	%	14	-0.134	-1.511	1.244	0.703	0.849	7.547	171.532	< 0.001	90.672
IVOMD	%	8	1.298	-1.523	4.120	1.439	0.367	15.525	153.287	< 0.001	95.433
Rumen fermentation											
pH		75	1.505	1.047	1.963	0.234	< 0.001	3.305	489.127	< 0.001	84.871
Ammonia	mg/dL	97	-1.782	-2.256	-1.309	0.241	< 0.001	4.599	713.864	< 0.001	86.552
Acetate	mol/100 mol	98	0.898	0.549	1.248	0.178	< 0.001	2.373	476.614	< 0.001	79.648
Propionate	mol/100 mol	98	-0.777	-1.152	-0.402	0.191	< 0.001	2.788	539.030	< 0.001	82.005
Butyrate	mol/100 mol	86	0.335	0.011	0.659	0.165	0.043	1.723	363.130	< 0.001	76.592
iso-Butyrate	mol/100 mol	46	-0.425	-0.892	0.041	0.238	0.074	1.906	184.843	< 0.001	75.655
Valerate	mol/100 mol	53	-0.369	-0.745	0.007	0.192	0.054	1.272	162.107	< 0.001	67.922
iso-Valerate	mol/100 mol	46	-0.677	-1.121	-0.233	0.226	0.003	1.664	168.556	< 0.001	73.303
A/P		90	1.175	0.774	1.576	0.205	< 0.001	2.934	513.168	< 0.001	82.657
Total VFA	mM	159	-3.007	-3.434	-2.580	0.218	< 0.001	6.146	1336.026	< 0.001	88.174
Gas production 24 h	ml/g OM	48	-3.531	-4.426	-2.635	0.457	< 0.001	8.463	536.982	< 0.001	91.247
Gas production 48 h	ml/g OM	28	-3.365	-4.167	-2.563	0.409	< 0.001	3.681	163.534	< 0.001	83.49

Note: A/P = acetate: propionate ratio; VFA = volatile fatty acid; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility

Fatty acid profile and rumen BH activity

In Table 4, the supplementation of essential oils and their derivatives is shown to have a very significant effect ($P < 0.001$) on the proportion of several rumen beneficial fatty acids, namely C18:1 n-9, C18:2 n-6, CLA c9 t11, C18:2 n-6, C18:3 n-3, C20:5 n-3, and C22:6 n-3. Similar results were also shown in the MUFA and PUFA fatty acid groups. On the contrary, a very significant decrease ($P \leq 0.01$) was identified in the intermediate and final phase fatty acid products of rumen BH, namely C18:1 t, C18:1 t11, C18:0 and SFA, but not CLA t10 c12. The implication of increased accumulation of rumen fluid n-6 and n-3 fatty acids as the EOD dose increased had a highly significant ($P < 0.001$) inhibitory effect on the levels of BH C18:2 n-6, C18:3 n-3, C20:5 n-3 and C22:6 n-3, at 53.32, 61.71, 49.14 and 27.77% respectively (Table 4). The statistical value of I^2 rumen fatty acid and BH variables ranged from 59.762 - 93.434%, which is considered to be the level of heterogeneity appropriate for random effect models.

Meta-regression

The analysis showed that EOD supplementation was closely associated with the inhibitory effect of rumen BH. Increasing EOD dosage had a negative linear relationship with the BH activity of C18:2 n-6 (Figure 3; $P < 0.01$; $R^2 = 0.091$) and with the regression formula, namely Y=-2.351 + -0.0211 x. BH C18:3 n-3 (Figure 4; $P \leq 0.001$; $R^2 = 0.103$); with the reduction equation Y=-2.598 + -0.0180 x. BH C20:5 n-3 (Figure 5; $P < 0.001$; $R^2 = 0.171$); with a linear equation Y= 0.0695 + -0.0243 x and BH C22:6 n-3 (Figure 6; $P < 0.05$; $R^2 = 0.061$); with the equation formula Y=-1.305 + -0.00910 x.

Publication bias

Detection of publication or study bias integrated into the meta-analysis database through the adjusted selection model method shows non-significant results ($P > 0.05$), or there is no publication bias in the total VFA as a primary parameter in rumen anaerobic digestion. The weight function plot as a visual assessment is presented in Figure 7.

Table 4 Effect of EOD supplementation on *in vitro* rumen fatty acid profile and BH activity

Variable	n	Estimate	Lower bound	Upper bound	Std. error	P-Value	τ^2	Q	Het. P-value	I^2
Fatty acid profile (g/ 100 g TFA)										
C14:0	48	-0.139	-0.503	0.225	0.186	0.453	1.162	189.902	< 0.001	75.250
C16:0	82	-0.868	-1.274	-0.462	0.207	< 0.001	2.664	458.588	< 0.001	82.337
C16:1	33	1.698	1.030	2.366	0.341	< 0.001	3.150	243.496	< 0.001	86.858
C18:0	162	-2.144	-2.695	-1.593	0.281	< 0.001	9.371	1911.994	< 0.001	91.579
C18:1 n-9	78	3.381	2.727	4.035	0.334	< 0.001	6.727	560.369	< 0.001	86.259
C18:1 t	34	-4.415	-5.537	-3.293	0.572	< 0.001	8.091	189.232	< 0.001	82.561
C18:1 t11	183	-0.588	-1.038	-0.138	0.230	0.010	7.089	1906.383	< 0.001	90.453
C18:2 n-6	192	4.767	4.213	5.320	0.282	< 0.001	11.340	2313.339	< 0.001	91.744
CLA c9 t11	199	0.809	0.547	1.072	0.134	< 0.001	2.763	1198.724	< 0.001	83.482
CLA t10 c12	59	0.088	-0.231	0.407	0.163	0.590	0.834	144.142	< 0.001	59.762
C18:3 n-3	191	5.549	4.957	6.142	0.302	< 0.001	12.588	2392.511	< 0.001	92.059
C20:5 n-3	118	8.107	7.276	8.939	0.424	< 0.001	14.922	1425.290	< 0.001	91.791
C22:6 n-3	118	4.745	4.175	5.315	0.291	< 0.001	7.226	1055.11	< 0.001	88.911
SFA	51	-0.800	-1.293	-0.308	0.251	0.001	2.668	343.422	< 0.001	85.441
MUFA	48	2.633	1.868	3.398	0.390	< 0.001	6.256	516.473	< 0.001	90.900
PUFA	48	4.929	3.837	6.021	0.557	< 0.001	11.052	715.790	< 0.001	93.434
Biohydrogenation activity (%)										
C18:2 n-6	100	-4.990	-5.648	-4.332	0.336	< 0.001	8.677	1076.834	< 0.001	90.806
C18:3 n-3	102	-4.959	-5.612	-4.306	0.333	< 0.001	8.800	1118.012	< 0.001	90.877
C20:5 n-3	78	-4.630	-5.276	-3.984	0.330	< 0.001	6.643	668.380	< 0.001	88.480
C22:6 n-3	78	-2.963	-3.456	-2.469	0.252	< 0.001	3.993	547.279	< 0.001	85.930

Note: CLA = conjugated linoleic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid

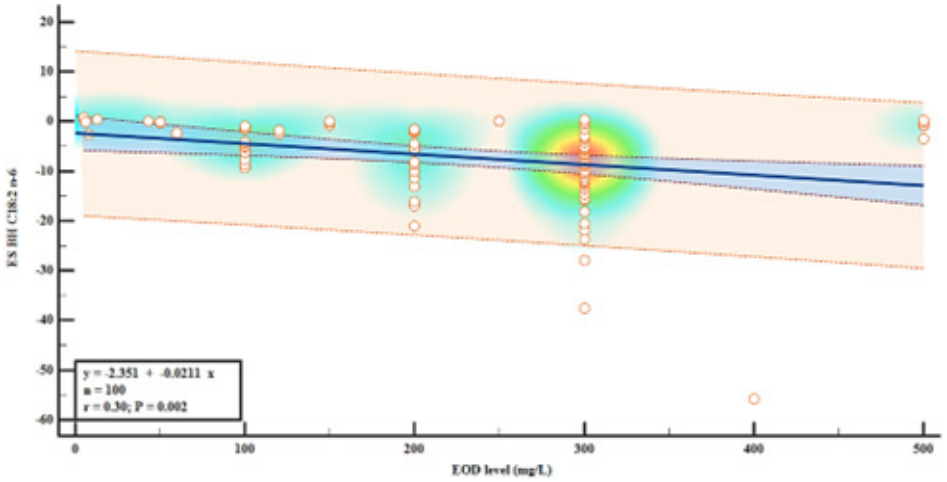

Figure 3 Meta-regression plot of *in vitro* BH C18:2 n-6.

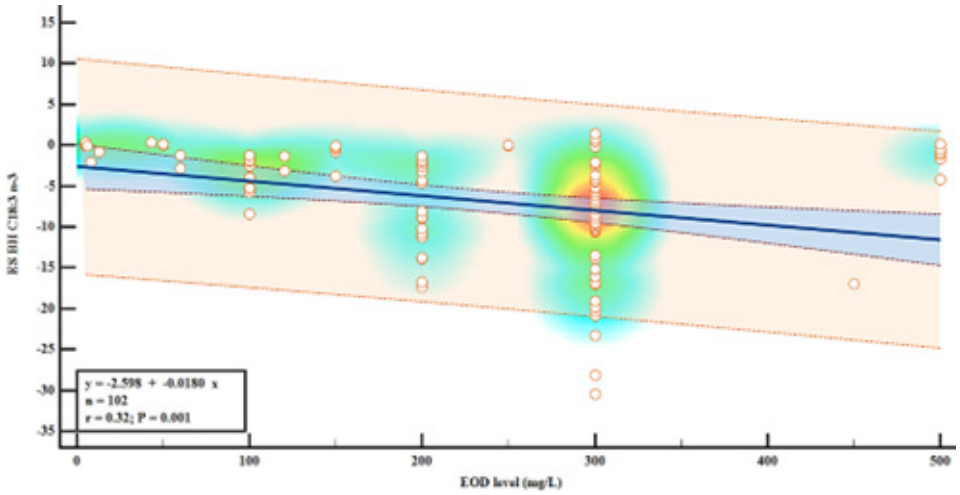

Figure 4 Meta-regression plot of *in vitro* BH C18:3 n-3.

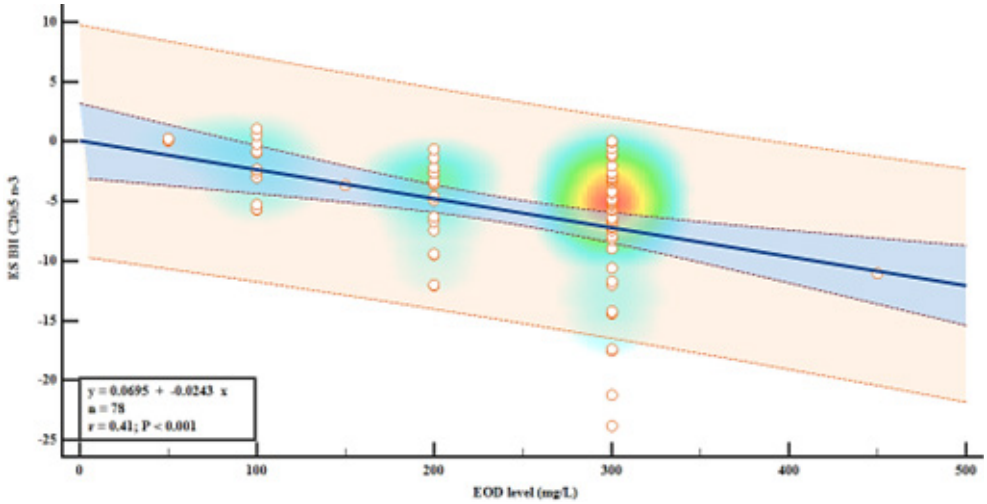

Figure 5 Meta-regression plot of *in vitro* BH C20:5 n-3.

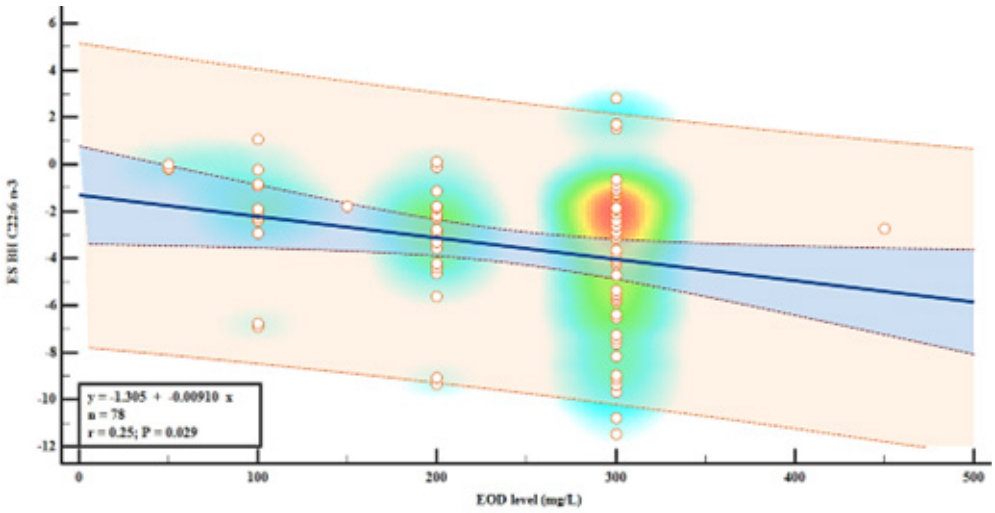

Figure 6 Meta-regression plot of *in vitro* BH C22:6 n-3.

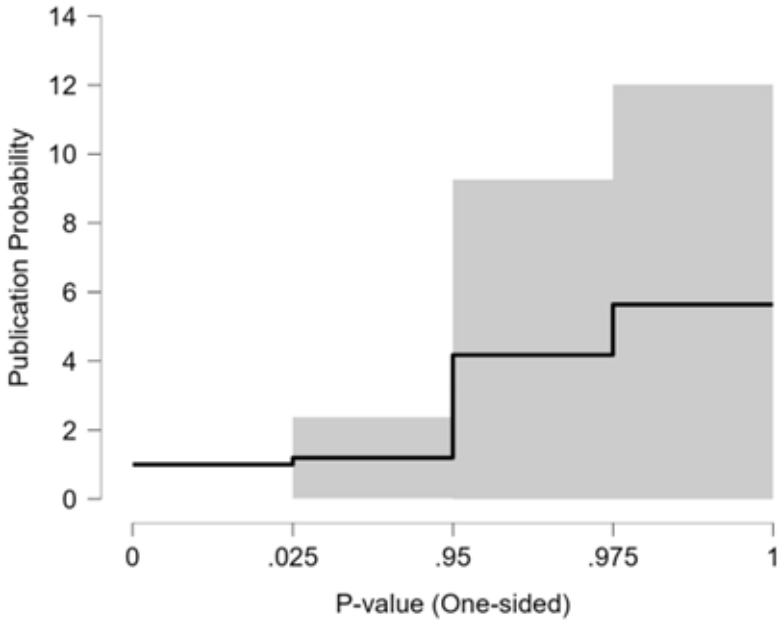

Figure 7 Weight function plot for total VFA.

DISCUSSION

EOD supplementation as a natural rumen additive showed the effect of increasing pH in the rumen environment, but this was still within the ideal range. Changes in rumen fluid pH have been known to influence rumen PUFA BH patterns significantly. This is certainly related to the high sensitivity of rumen microbial populations and species associated with various phases of lipid metabolism to changes in acidity in the rumen environment. In addition, this is also a direct effect of the strong antimicrobial properties of EOD that modulate VFA production, nitrogen metabolism, and the rumen microbial community (Calsamiglia et al., 2007; Kim et al., 2019). Troegeler-Meynadier et al. (2003) confirmed the effect of high pH buffers (6.4 - 6.9) on increasing

the concentration of the intermediate fatty acid C18:2 c9 t11 during the 24 h incubation period. However, this was not the case for other types of intermediary fatty acids, as an increase in pH from 5.5 to 6.5 showed a decrease in CLA c10 c12 concentrations (Lee, 2013). In addition, the negative relationship between the production of C18:0 with some branched-chain fatty acids and IVOMD is not yet known. To date, the bacterium *Butyrivibrio proteoclasticus* is the only microbe known to act as a producer of C18:0 and butyrate from the terminal phase of BH. Nevertheless, it does not rule out the involvement of other microbes (Huws et al., 2010). Genomic analysis of the rumen of dairy cows characterised by milk high in saturated fatty acids showed an association with the abundance of several rumen microbial communities, namely lactic acid bacteria, acetogenic proteobacteria, and fungi (Stergiadis et al., 2021).

In general, EO affects the characteristics of rumen fermentation in relation to several aspects, namely: 1) increasing cation transport; 2) inhibiting proteolysis activity; 3) decreasing ammonia production; 3) modulating glutamine production; and 4) inhibiting the growth of microbial methanogens (Nehme et al., 2021). Elevated cation transport in the rumen system may correlate with the mode of action of some EOD such as carvacrol that acts as transmembrane carriers of monovalent cations, where the exchange of hydroxyl protons with cations (K^+) occurs (Bodas et al., 2012). The broad-spectrum antimicrobial effect of the EO group has an impact on the reduction of proteolytic bacteria, where EO exposure causes disruption of bacterial cytoplasmic membrane and leads to a decrease in protein degradation as well as ammonia concentration (Ranilla et al., 2023). The effect of EO on increasing acetic acid production and reducing the rumen ammonia concentration of various types of aromatic oils was also observed by Benetel et al. (2022), with *Thymus vulgaris* oil (500 mg/L) causing an increase in the acetate proportion of 1.8%, a decrease in ammonia concentration of 31.9% and a reduction in IVDMD of 24.8%. In comparison, thymol supplementation showed a significant decrease in VFA, ammonia, IVDMD, and IVOMD concentrations at a dose of 400 mg/L (Yu et al., 2020). This indicates the suppression of rumen microbial fermentation activity with EOD as a dietary additive. Patra (2011) explained that low doses of EO can selectively suppress population growth and substrate availability for amylolytic, proteolytic, and hyper ammonia-producing bacteria without affecting fibre degradation as a precursor to the formation of acetic acid and butyrate. At higher doses (986 mg/L), the EOD type bornyl acetate showed no significant decrease in VFA concentration, but effectively reduced methane production *in vitro* (Joch et al., 2016). Therefore, the effect of the addition of EOD on the metabolic process of rumen nutrients shows a varied response. This is because the effect is highly dependent on the EOD source, composition, and dose. Abiotic factors such as altitude and soil type are reported to have an influence on changes in the chemical composition of *Thymus* EO (Gherairia et al., 2022). A mixture of EO sourced from lemongrass and coriander showed a synergistic effect on rumen metabolic parameters, but there was an antagonistic effect when ginger oil was added (Temmar et al., 2021). Cobellis et al. (2015) reported the effect of *Rosmarinus officinalis* EO (1.75%), which tended to reduce the total rumen microbial population and cellulolytic, but not amylolytic. The mode of action of *Citrus medica* EO (45.36% limonene and 21.23% γ-terpinene) showed an effect of disrupting the integrity and permeability of

cellular membranes of gram-positive and gram-negative bacteria, characterised by the leakage of nucleic acid components after exposure for 7-12 hours (Li et al., 2019). Some types of EO showed higher inhibitory efficacy on gram negative bacteria (Al-Shuneigat et al., 2015). A modelling approach to the antibacterial action of *Croton blanchetianus* EO showed a pattern of growth reduction of *Escherichia coli* and inactivation of *Listeria monocytogenes* (de Vasconcelos et al., 2022).

The results of a meta-analysis of 74 publications on *in vivo* experiments in small ruminants showed an insignificant effect on acetate and butyrate production as the dose of EO inclusion increased (<500 to >1000 mg/kg DM), but significantly reduced the formation of rumen ammonia (Dorantes-Iturbide et al., 2022). Therefore, EO supplementation has more impact on reducing ammonia gas emissions when compared to methane and carbon dioxide (Carrazco et al., 2020). In addition, evaluating oregano EO at doses of 150 ppm and 0.5 mg/L showed a real reduction in IVDMD and gas production parameters on various test substrates (Kilic et al., 2011; Righi et al., 2017). Conversely, adding EO doses of clove, mint, and cinnamon of up to 300 ppm showed increased rumen organic matter digestibility *in vitro* (Rofiq et al., 2021). In addition, EO have a stronger toxic effect than condensed tannin on rumen methanogens (Cieslak et al., 2013), 0.50% orange EO (78.84% D-Limonene) contributed to a 12% reduction in ruminant methane production (Jiménez-Ocampo et al., 2022).

EOD supplementation as a BH inhibitory agent is highly evident from the decrease in the accumulation of C18:0 as the main product of rumen lipid metabolism, along with an increase in bioactive fatty acids such as CLA c9 t11, which is an intermediate product of BH. Previous studies have shown that higher CLA c9 t11 is due to anise oil supplementation at a level of 200 mg/L facilitates linoleate isomerase activity in forming CLA c9 t11 from c9 c12 C18:2 in the first step of BH (Eburu et al., 2017). This also indicates a specific inhibitory mechanism in late phases of rumen lipid metabolism. Infusion of garlic EO (0.8 g/d) did not significantly affect the population of *Butyrivibrio* and *B. proteoclasticus* bacterial groups in the rumen of goats (Zhu et al., 2012). Furthermore, garlic EO derivatives, namely diallyl disulfide and propyl propane thiosulfinate, have been reported not to restrain the growth of the *Butyrivibrio* bacterial group (Ramos-Morales et al., 2013). The same results were confirmed by Miri et al. (2015), who utilised cumin seed extract (*Cuminum cyminum*), which showed no inhibitory effect on the growth of the rumen bacteria that play a role in the early phase of BH, namely *Butyrivibrio hungatei*, *Butyrivibrio fibrisolvens* and *B. proteoclasticus* bacteria, which play a role in the late phase. However, lipase activity was detected to be reduced in *B. proteoclasticus* strain B316 as the EO dose was increased. EO also reported to have strong inhibitory activity against α-glucosidase enzyme (Benouchenne et al., 2022). The block of the enzyme activity might lead to the modulation of rumen PUFA BH. Little is known about the metabolic processes on the C20 and C22 fatty acid groups of rumen PUFA. However, EOD additives contribute to the proportion of these fatty acid groups, similar to the C18 PUFA group. Sakurama et al. (2014) showed that the anaerobic bacterium *Clostridium bifermentans* JCM 1386 was able to convert C20:5 n-3 (eicosapentaenoic acid or EPA) to C20:4 n-7 (eicosatetraenoic acid). There are indications that bacteria of this species are found in the rumen fibrolytic bacterial community

of swamp buffaloes (Boonsaen et al., 2010). Compared to the median level of BH EPA at 69% from microalgae biomass *Nannochloropsis oceanica* after 24 h incubation (Alves et al., 2018), the level of protection displayed by EOD remained superior. Furthermore, BH of C22:6 n-3 (docosahexaenoic acid or DHA) to C22:0 (docosanoic acid) involves the bacteria *B. proteoclasticus* P18, *Acetobacter* and *Bacillus* (Jeyanathan et al., 2016; Huang et al., 2020). Aldai et al. (2018) reported 80% of BH DHA at 6 hours of incubation. The average BH of EPA and DHA reported through *in vivo* rumen evaluations was 91% and 89% (Scollan et al., 2001).

The effective dose of EOD in suppressing BH long-chain PUFA in this meta-analysis study was 300 mg/L. This can be observed from the meta-regression analysis of each n-6 and n-3 BH activity, where most of the effect sizes accumulated at these levels. The significant effect of EOD in suppressing rumen BH activity is accompanied by disruption of rumen fermentation activity, so the administration level must be considered. Calsamiglia et al. (2007) recommend safe levels of EOD additive supplementation in the rumen environment ranging from 50 to 500 mg/L. In addition, the results also confirm EOD superior effect compared to tannins in suppressing BH C18 PUFA activity *in vitro* (Makmur et al., 2022). EOD supplementation is expected to be one of the promising strategies for improving fatty acid profiles and realising functional foods from animal sources. Intake of C18:3 n-3, C20:5 n-3, C22:5 n-3, and C22:6 n-3 in the human diet has been reported to reduce the risk of cancer and cardiovascular disease (Nguyen et al., 2019).

The positive effect of EOD addition can be seen from the abundant proportion of PUFA and CLA groups in the rumen fluid. These results are expected to promote the transfer of beneficial fatty acid deposits in ruminant-derived products. Lydia et al. (2020) reported an increase in n-3 PUFA in the subcutaneous fat of cattle supplemented with a 1 g/head/day blend of EO (eugenol, limonene, thymol, vanillin, and guaiacol). However, the increase showed insignificant results. This may be due to the ability of rumen microbes to degrade the bioactive components of EO, or the formation of resistance mechanisms from rumen microbes to the biological activity of these components (Szumacher-Strabel et al., 2015). The study by Zhang et al. (2022) showed that oregano EO (130 mg/d) was able to improve the fatty acid profile and organoleptic and increase the volatile components of Pingliang red cattle semitendinosus muscle.

Furthermore, *Arnica montana* EO additive (450-1350 mg EO/kg DM) reduced the C18:1 t11 content of (Dias et al., 2022). In addition, using EO as an additive for dairy cattle increased PUFA content, milk-free fatty acids, and total milk yield (Giannenas et al., 2011; Köknur et al., 2021; Ghoneem and Mahmoud 2022). In addition, Khiaosa-ard and Zebeli (2013) reported the results of a meta-analysis of 28 publications on the effects of adding EO and their bioactive compounds on improving milk protein content and composition.

CONCLUSIONS

This meta-analysis has shown that EOD supplementation can inhibit the formation of ammonia, propionate, *iso*-valerate, total VFA, and rumen gas production *in vitro*. In addition, it can increase the proportion of C18:1 n-9, CLA c9 t11, C18:2 n-6, C18:3 n-3, C20:5 n-3, C22:6 n-3, total MUFA, and PUFA in rumen fluid. Increasing the EOD level inhibited the activity of BH C18:2 n-6, C18:3 n-3, C20:5 n-3, and C22:6 n-3. It is expected that ruminant-derived products will be able to meet health standards regarding fatty acid profiles by using EOD as a source of natural additives. Further systematic review should be directed toward animal studies to confirm these findings.

ACKNOWLEDGEMENTS

Author thanks and appreciation to the head of research center for applied zoology BRIN, Dr. rer. nat. Evy Ayu Arida for helpful support. This work was supported by the National Research and Innovation Agency Republic of Indonesia [BRIN] under postdoctoral grant [code 151/II/HK/2022].

AUTHOR CONTRIBUTIONS

All authors contribute equally.

REFERENCES

Aldai, N., Delmonte, P., Alves, S.P., Bessa, R.J.B., Kramer, J.K.G., 2018. Evidence for the initial steps of DHA biohydrogenation by mixed ruminal microorganisms from sheep involves formation of conjugated fatty acids. J. Agric. Food. Chem. 66(4), 842-855.

Al-Shuneigat, J.M., Al-Tarawneh, I.N., Al-Qudah, M.A., Al-Sarayreh, S.A., Al-Saraireh, Y.M., Alsharafa, K.Y., 2015. The chemical composition and the antibacterial properties of Ruta graveolens L. essential oil grown in Northern Jordan. Jordan. J. Biol. Sci. 8(2), 139-143.

Alves, S.P., Mendonça, S.H., Silva J.L., 2018. Nannochloropsis oceanica, a novel natural source of rumen-protected eicosapentaenoic acid (EPA) for ruminants. Sci. Rep. 8, 10269.

Bartoš, F., Maier, M., Quintana, D.S., Wagenmakers, E., 2022. Adjusting for publication bias in JASP and R: selction models, pet-peese, robust bayesian meta-analysis. Adv. Meth. Pract. Psychol. Sci. 5(3), 1-19.

Benchaar, C., 2020. Feeding oregano oil and its main component carvacrol does not affect ruminal fermentation, nutrient utilization, methane emissions, milk production, or milk fatty acid composition of dairy cows. J. Dairy. Sci. 103(2), 1516-1527.

Benchaar, C., Calsamiglia, S., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed. Sci. Technol. 145(1–4), 209-228.

Benetel, G., Silva, T.D.S., Fagundes, G.M., Welter, K.C., Melo, F.A., Lobo, A.A.G., Muir, J.P., Bueno, I.C.S., 2022. Essential oils as in vitro ruminal fermentation manipulators to mitigate methane emission by beef cattle grazing tropical grasses. Molecules. 27, 2227.

Bodas, R., Prieto, N., García-González, R., Andrés, S., Giráldez, F.J., López, S., 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed. Sci. Technol. 176(1-4), 78-93.

Benouchenne, D., Belil, I., Bensouici, C., Yilmaz, M.A., Akkal, S., Keskinkaya, H.B., Khelifi, D., 2022. GC-MS chemical profile, antioxidant ability, antibacterial effect, a-glucosidase, a-amylase and acetylcholinesterase inhibitory activity of Algerian fir essential oil. Jordan. J. Biol. Sci. 15(2), 303-310.

Bokharaeian, M., Chikunya, S., 2012. Effects of 3-carene, resorcinol, and p-cresol on the metabolism of polyunsaturated fatty acids by rumen microorganisms in vitro. Adv. Anim. Biosci. 3(1), 106.

Boonsaen, P., Innuruk, P., Phiriyangkul. P., Sawanon, S., 2010. Isolation of a novel fibrolytic bacterium from the buffalo rumen phylogenetically closely related to Clostridium bifermentans. In Proceeding of international conference on biotechnology for healthy living. Prince of Songkhla University, Trang, Thailand. pp. 787–795.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J. Dairy. Sci. 90, 2580–2595.

Carrazco, A.V., Peterson, C.B., Zhao, Y., Pan, Y., McGlone, J.J., DePeters, E.J., Frank, M.M., 2020. The impact of essential oil feed supplementation on enteric gas emissions and production parameters from dairy cattle. Sustainability. 12(24), 10347.

Cieslak, A., Szumacher-Strabel, M., Stochmal, A., Oleszek, W., 2013. Plant components with specific activities against rumen methanogens. Animal. 7(Supplement 2), 253-265.

Cobellis, G., Acuti, G., Forte, C., Menghini, L., De Vincenzi, S., Orrù, M., Valiani, A., Pacetti, D., Trabalza-Marinucci, M., 2015. Use of Rosmarinus officinalis in sheep diet formulations: Effects on ruminal fermentation, microbial numbers and in situ degradability. Small. Rumin. Res. 126(Supplement 1), 10-18.

Daning, D.R.A., Hanim, C., Widyobroto, B.P., Yusiati, L.M., 2021. Characteristics of ruminal fatty acids using in vitro culture system by addition of galangal (Alpinia galangal). In Proceedings of the 9th international seminar on tropical animal production. Faculty of Animal Science UGM, Indonesia, 21–22 September 2021.

Daning, D.R.A., Yusiati, L.M., Hanim, C., Widyobroto, B.P., 2022. Dietary supplementation of galangal (Alpinia galangal) essential oil affects rumen fermentation pattern. Adv. Anim. Vet. Sci. 10(2), 323-334.

Daning, D.R.A., Hanim, C., Widyobroto, B.P., Yusiati, L.M., 2020. The use essential oil as rumen modifier in dairy cows. Wartazoa. 30(4), 189-200.

de Vasconcelos, E.C., Longhi, D.A., Paganini, C.C., Severo, D.S., Canuto, K.M., Souza, A.S.Q., Figueiredo, E.A.T., Aragão, G.M.F., 2022. Modeling the effect of Croton blanchetianus Baill essential oil on pathogenic and spoilage bacteria. Arch. Microbiol. 204, 618.

Dias, P.C.G., Ferreira, E.M.M., Santos, I.J., Carlis, M., Vicente, A.C.S., Silva, A.S., Assis, R.G., Comelli, J.H., Biava, J.S.S., Soares, L.C.B., Pires, A.V., 2022. PSVI-16 the essential oil of Arnica montana positively modulates the fatty acid profile of lamb meat. J. Anim. Sci. 100(Supplement 3), 403–404.

Dorantes-Iturbide, G., Orzuna-Orzuna, J.F., Lara-Bueno, A., Mendoza-Martínez, G.D., Miranda-Romero, L.A., Lee-Rangel, H.A., 2022. Essential oils as a dietary additive for small ruminants: a meta-analysis on performance, rumen parameters, serum metabolites, and product quality. Vet. Sci. 9, 475.

Doreau, M., Arturo-Schaan, M., Laverroux, S., 2017. Garlic oil reduces ruminal fatty acid biohydrogenation in vitro. Eur. J. Lipid Sci. Technol. 119(4), 1500388.

Eburu, P.O., Anya, M.I., 2020. Dose-response effects of anise essential oil on rumen fermentation and metabolism of n-3 polyunsaturated fatty acids in vitro. Asian. J. Anim. Vet. Adv. 15, 25-31.

Eburu, P.O., Anya, M.I., 2018. The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African dwarf goats. Online J. Anim. Feed Res. 8(5), 126-135.

Eburu, P.O., Chikunya, S., 2014. Assessment of the effects of fifteen compounds from essential oil extracts on the metabolism of polyunsaturated fatty acids by rumen microorganisms in vitro. Adv. Anim. Biosci. 5(1), 78.

Eburu, P.O., Chikunya, S., 2015a. Effects of ten whole essential oils on rumen fermentation and biohydrogenation of n-3 polyunsaturated fatty acids by rumen microorganisms in vitro. Adv. Anim. Biosci. 6(2), 203.

Eburu, P.O., Chikunya, S., 2015b. The effects of graded doses of 4-allylanisole, anethole, anise oil and cassia oil on fermentation and biohydrogenation of n-3 polyunsaturated fatty acids by rumen microorganisms in vitro. Adv. Anim. Biosci. 6(2), 204.

Eburu, P.O., Chikunya, S., Anya, M.I., Ozung, P.O., 2017. Effects of rumen exposure to anise oil on ruminal fermentation and biohydrogenation of n-3 polyunsaturated fatty acids. Glob. J. Pure. Appl. Sci. 23(2), 231-243.

Foskolos, A., Siurana, A., Rodriquez-Prado, M., Ferret, A., Bravo, D., Calsamiglia, S., 2015. The effects of a garlic oil chemical compound, propyl-propane thiosulfonate, on ruminal fermentation and fatty acid outflow in a dual-flow continuous culture system. J. Dairy. Sci. 98(8), 5482-5491.

Gherairia, N., Boukerche, S., Mustapha, M.A., Chefrour, A., 2022. Effects of biotic and abiotic factors on the yield and chemical composition of essential oils from four Thymus species wild-growing in Northeastern Algeria. Jordan J. Biol. Sci. 15(2), 173-181.

Ghoneem, W.M.A., Mahmoud, A.E.M., 2022. Impact of incorporating Thymus vulgaris as leaves or essential oil in Damascus goats ration on lactation performance. Int. J. Dairy Sci. 17, 1-12.

Giannenas, I., Skoufos, J., Giannakopoulos, C., Wiemann, M., Gortzi, O., Lalas, S., Kyriazakis, I., 2011. Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. J. Dairy. Sci. 94(11), 5569-5577.

Gilman, F., Chikunya, S., 2014. The effects of whole clove bud oil or its three principal constituent components on the biohydrogenation of polyunsaturated fatty acids by rumen microorganisms in vitro. Adv. Anim. Biosci. 5(1), 84.

Guerreiro, O., Alves, S.P., Costa, M., Cabo, Â., Duarte, M.F., Jerónimo, E., Bessa, R.J.B., 2016. Effects of extracts obtained from Cistus ladanifer L. on in vitro rumen biohydrogenation. Anim. Feed. Sci. Technol. 219, 304-312.

Gunal, M., Ishlak, A., Abughazaleh, A.A., 2013. Evaluating the effects of six essential oils on fermentation and biohydrogenation in in vitro rumen batch cultures. Czech J. Anim. Sci. 558(6), 243-252.

Gunal, M., Ishlak, A., AbuGhazaleh, A.A., Khattab, W., 2014. Essential oil effect on rumen fermentation and biohydrogenation under in vitro conditions. Czech. J. Anim. Sci. 59(10), 450-459.

Gurevitch, J., Nakagawa, S., 2015. Research synthesis methods in ecology. In: Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J. (Eds), Ecological Statistics: Contemporary theory and application. Oxford Academic, Oxford.

Huang,G.,Zhang,Y.,Xu,Q.,Zheng,N.,Zhao,S.,Liu,K.,Qu,X.,Yu,J.,Wang,J.,2020.DHAcontentin milk and biohydrogenation pathway in rumen: a review. PeerJ. 8, e10230.

Huws, S.A., Lee, M.R.F., Muetzel, S.M., Scott, M.B., Wallace, R.J., Scollan, N.D., 2010. Forage type and fish oil cause shifts in rumen bacterial diversity. FEMS. Microbiol. Ecol. 73, 396–407.

Ishlak, A., Günal, M., AbuGhazaleh, A.A., 2015. The effects of cinnamaldehyde, monensin and quebracho condensed tannin on rumen fermentation, biohydrogenation and bacteria in continuous culture system. Anim. Feed. Sci. Technol. 207, 31-40.

Jeyanathan, J., Escobar, M., Wallace, R.J., Fievez, V., Vlaeminck, B., 2016. Biohydrogenation of 22:6 n-3 by Butyrivibrio proteoclasticus P18. BMC. Microbiol. 16, 104.

Jiménez-Ocampo, R., Montoya-Flores, M.D., Pamanes-Carrasco, G., Herrera-Torres, E., Arango, J., Estarrón-Espinosa, M., Aguilar-Pérez, C.F., Araiza-Rosales, E.E., Guerrero-Cervantes, M., Ku-Vera, J.C., 2022. Impact of orange essential oil on enteric methane emissions of heifers fed bermudagrass hay. Front. Vet. Sci. 9, 863910.

Joch, M., Cermak, L., Hakl, J., Hucko, B., Duskova, D., Marounek, M., 2016. In vitro screening of essential oil active compounds for manipulation of rumen fermentation and methane mitigation. Anim. Biosci. 29(7), 952-959.

Khiaosa-ard, R., Zebeli, Q., 2013. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. J. Anim. Sci. 91(4), 1819–1830.

Kilic, U., Boga, M., Gorgulu, M., Şahan, Z., 2011. The effects of different compounds in some essential oils on in vitro gas production. J. Anim. Feed. Sci. 20(4), 626-636.

Kim, H., Jung, E., Lee, H.G., Kim, B., Cho, S., Lee, S., Kwon, I., Seo, J., 2019. Essential oil mixture on rumen fermentation and microbial community – an in vitro study. Asian-Australas J. Anim. Sci. 32(6), 808-814.

Köknur, Ö., Beyzi, S.B., Konca, Y., 2021. Effects of dietary essential oil and live yeast supplementation on dairy performance, milk quality and fatty acid composition of dairy cows. Large Anim. Rev. 28, 15-20.

Lee, Y., 2013. Effect of pH on conjugated linoleic acid (CLA) formation of linolenic acid biohydrogenation by ruminal microorganisms. J. Microbiol. 51(4), 471–476.

Li, Z.H., Cai, M., Liu, Y.S., Sun, P.L., Luo, S.L., 2019. Antibacterial activity and mechanisms of essential oil from Citrus medica L. var. sarcodactylis. Molecules. 24, 1577.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleijenen, J., Moher, D., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 339, b2700.

Lourenço, M., Cardozo, P.W., Calsamiglia, S., Fievez, V., 2008. Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. J. Anim. Sci. 86(11), 3045–3053.

Lourenço, M., Falchero, L., Tava, A., Fievez, V., 2009. Alpine vegetation essential oils and their effect on rumen lipid metabolism in vitro. In Proceedings of the XIth international symposium on ruminant physiology. Wageningen Academic Publishers, Wageningen.

Lydia, M.W., Huang, S., Chalupa-Krebzdak, S., Mejía, S.M.V., Mandell, I.B., Bohrer, B.M., 2020. Effects of essential oils and(or) benzoic acid in beef finishing cattle diets on the fatty acid profile and shelf life stability of ribeye steaks and ground beef. Meat. Sci. 168, 108195.

Makmur, M., Zain, M., Sholikin, M.M., Suharlina, Jayanegara, A., 2022. Modulatory effects of dietary tannins on polyunsaturated fatty acid biohydrogenation in the rumen: a meta-analysis. Heliyon. 8(7): e09828.

Mandal, G.P., Roy, A., Patra, A.K., 2016. Effects of plant extracts rich in tannins, saponins and essential oils on rumen fermentation and conjugated linoleic acid concentrations in vitro. Indian J. Anim. Hlth. 55(1), 49-60.

Miri, V.H., Ebrahimi, S.H., Tyagi, A.K., 2015. The effect of cumin (Cuminum cyminum) seed extract on the inhibition of PUFA biohydrogenation in the rumen of lactating goats via changes in the activity of rumen bacteria and linoleate isomerase enzyme. Small. Rumin. Res. 125, 56-63.

Nanon, A., Suksombat, W., Yang, W.Z., 2014. Effects of essential oils supplementation on in vitro and in situ feed digestion in beef cattle. Anim. Feed Sci. Technol. 196, 50-59.

Nehme, R., Andrés, S., Pereira, R.B., Ben Jemaa, M., Bouhallab, S., Ceciliani, F., López, S., Rahali, F.Z., Ksouri, R., Pereira, D.M., Abdennebi-Najar, L., 2021. Essential oils in livestock: from health to food quality. Antioxidants. 10, 330.

Nguyen, Q.V., Malau-Aduli, B.S., Cavalieri, J., Nichols, P.D., Malau-Aduli, A.E.O., 2019. Enhancing omega-3 long-chain polyunsaturated fatty acid content of dairy-derived foods for human consumption. Nutrients. 11(4), 743.

Patra, A.K., 2011. Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. Asian. J. Anim. Vet. Adv. 6, 416-428.

Purba, R.A.P., Yuangklang, C., Paengkoum, P., 2020. Enhanced conjugated linoleic acid and biogas production after ruminal fermentation with Piper betle L. supplementation. Ciênc. Rural. 50(7), e20191001.

Ramos-Morales, E., Martínez-Fernández, G., Abecia, L., Martin-García, A.I., Molina-Alcaide, E., Yáñez-Ruiz, D.R., 2013. Garlic derived compounds modify ruminal fatty acid biohydrogenation and induce shifts in the community in continuous-culture fermenters. Anim. Feed. Sci. Technol. 184(1–4), 38-48.

Ranilla, M.J., Andrés, S., Gini, C., Biscarini, F., Saro, C., Martín, A., Mateos, I., López, S., Javier F.G., Abdennebi-Najar, L., Pereira, D., Falleh H., Ksouri, R., Cremonesi, P., Castiglioni, B., Ceciliani, F., 2023. Effects of Thymbra capitata essential oil on in vitro fermentation end-products and ruminal bacterial communities. Sci. Rep. 13, 4153.

Righi, F., Simoni, M., Foskolos, A., Beretti, V., Sabbioni, A., Quarantelli, A., 2017. In vitro ruminal dry matter and neutral detergent fibre digestibility of common feedstuffs as affected by the addition of essential oils and their active compounds. J. Anim. Feed. Sci. 26(3), 204-212.

Rofiq, M.N., Negara, W., Martono, S., Gopar, R.A., Boga, M., 2021. Potential effect of some essential oils on rumen methane reduction and digestibility by in vitro incubation technique. IOP Conf. Ser.: Earth. Environ. Sci. 905, 012138.

Sakurama, H., Kishino, S., Mihara, K., Ando, A., Kita, K., Takahashi, S., Shimizu, S., Ogawa, J., 2014. Biohydrogenation of C20 polyunsaturated fatty acids by anaerobic bacteria. J. Lipid. Res. 55(9), 1855-1863.

Schoonjans, F., 2017. MedCalc manual: easy-to-use statistical software. Independently published. pp. 382.

Scollan, N., Dhanoa, M., Choi, N., Maeng, W., Enser, M., Wood, J., 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agric. Sci. 136(3), 345-355.

Sgwane, T.S., 2014. Effect of essential oil compounds on metabolism of dietary polyunsaturated fatty acids by ruminal microorganisms in vitro (Doctor of Philosophy Thesis). University of Aberdeen, Scotland, United Kingdom.

Siurana, A., Ferret, A., Rodriguez, M., Vlaeminck, B., Fievez, V., Calsamiglia, S., 2018. Strategies to modify the ruminal biohydrogenation of polyunsaturated fatty acids and the production of trans-10, cis-12 C18:2 in vitro. Anim. Feed Sci. Technol. 235, 158-165.

Stergiadis, S., Cabeza-Luna, I., Mora-Ortiz, M., Stewart, R.D., Dewhurst, R.J., Humphries, D.J., Watson, M., Roehe, R., Auffret, M.D., 2021. Unravelling the role of rumen microbial communities, genes, and activities on milk fatty acid profile using a combination of omics approaches. Front. Microbiol. 11, 590441.

Szumacher-Strabel, M., El-Sherbiny, M., Cieslak, A., Szczechowiak, J., Winiarska, H., 2015. Bioactive lipid components from ruminant milk and meat. Chapter 25. In: Biotechnology of bioactive compounds: sources and applications. Editors: Vijai K. Gupta, Maria Tuohy, Anthonia O'Donovan, Mohtashim Lohani. John Wiley & Sons. UK. pp. 599–629.

Temmar, R., Rodríguez-Prado, M., Forgeard, G., Rougier, C., Calsamiglia, S., 2021. Interactions among natural active ingredients to improve the efficiency of rumen fermentation in vitro. Animals. 11(5), 1205.

Tiven, N.C., 2017. Minimize the hydrogenation of unsaturated fatty acid in the rumen with kaffir lime (Citrus hystrix) leaves. Bull. Anim. Sci. 41(3), 265-270.

Troegeler-Meynadier, A., Nicot, M.C., Bayourthe, C., Moncoulon, R., Enjalbert, F., 2003. Effects of pH and concentrations of linoleic and linolenic acids on extent and intermediates of ruminal biohydrogenation in vitro. J. Dairy. Sci. 86, 4054–4063.

Ye, D., Karnati, S.K.R., Wagner, B., Firkins, J.L., Eastridge, M.L., Aldrich, J.M., 2018. Essential oil and monensin affect ruminal fermentation and the protozoal population in continuous culture. J. Dairy. Sci. 101(6), 5069-5081.

Yu, J., Cai, L., Zhang, J., Yang, A., Wang, Y., Zhang, L., Guan, L.L., Qi, D., 2020. Effects of thymol supplementation on goat rumen fermentation and rumen microbiota in vitro. Microorganisms. 8(8), 1160.

Zhang, R., Bai, Y., Jia, L., Sun, J., Li, J., Kong, F., Wang, L., Zhang, X., Wang, Y., Liu, T., Cheng, Q., Zhang, X., Zhang, K., Wu, J., Zhao, S., Lei, Z., 2022. Effects of oregano essential oil on meat quality, fatty acids and volatile flavor compounds in semitendinosus of pingliang red cattle. Chinese J. Anim. Nutr. 34(7), 4452-4463.

Zhu, Z., Mau, S., Zhu, W., 2012. Effects of ruminal infusion of garlic oil on fermentation dynamics, fatty acid profile and abundance of bacteria involved in biohydrogenation in rumen of goats. Anim. Biosci. 25(7), 962-970.

How to cite this article;

Malik Makmur, Yulianri Rizki Yanza, Ainissya Fitri, Syarifuddin, Roni Ridwan and Anuraga Jayanegara. Effects of essential oils and their derivatives on rumen fermentation characteristics and PUFA biohydrogenation: A meta-analysis of *in vitro* studies. Veterinary Integrative Sciences. 2023; 21(3): 925 - 944.