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ORIGINAL PAPER

Effects of thyme, lavender, and peppermint essential oils on ruminal biohydrogenation in fish oil-supplemented diets: A mechanistic *in vitro* study

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Short title: Essential oil supplementation and ruminal biohydrogenation

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ABSTRACT. Modifying ruminal biohydrogenation (BH) is a widely investigated approach to improve the fatty acid (FA) profile of ruminant-derived products. This study evaluated the effects of thyme (*Thymus vulgaris*; TEO), peppermint (*Mentha piperita*; PEO), and lavender (*Lavandula angustifolia*; LEO) essential oils (EOs) on *in vitro* ruminal short-chain fatty acid (SCFA) profiles and BH of polyunsaturated fatty acids (PUFAs). A basal substrate consisting of alfalfa hay and a concentrate mixture (60:40) supplemented with 2% fish oil (FO) was used as a source of long-chain PUFAs. The treatments included a control (FO-supplemented TMR without EO) and three EO groups, each added at 1.5 µl/ml of buffered rumen fluid. All EOs significantly inhibited the final step of BH, as indicated by a marked reduction in stearic acid (C18:0) concentration ($P < 0.01$). TEO supplementation resulted in the highest concentrations of c9, t11-CLA, and linoleic acid, suggesting inhibition of the final BH step and greater availability of vaccenic acid for endogenous CLA synthesis. Conversely, PEO resulted in the highest concentrations of eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) ($P < 0.01$), together with the lowest concentration of palmitic acid (C16:0),

indicating greater protection of fish oil-derived PUFAs. TEO also increased the acetate-to-propionate ratio ($P < 0.01$). Our findings demonstrate that the effects of the essential oils on ruminal BH differed between plant species. TEO promoted CLA accumulation, whereas PEO more effectively preserved EPA and DHA and reduced palmitic acid concentration.

Keywords: CLA; fish oil; essential oils; PUFA; rumen fermentation; ruminal biohydrogenation.

Introduction

Improving the nutritional value and fatty acid profile of ruminant-derived products has long been an important objective in animal nutrition research (Givens and Shingfield, 2004). Recent meta-analyses have indicated that increasing the proportion of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) improves the nutritional quality of these products and addresses the growing demand for foods produced using sustainable production systems (Frutos et al., 2020). However, dietary PUFAs undergo extensive biohydrogenation (BH), during which rumen microorganisms convert them into intermediate products and, ultimately, into SFAs (Kepler and Tove, 1967). As a result, only a limited proportion of beneficial PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reaches the lower digestive tract. Therefore, modifying specific steps of ruminal biohydrogenation (BH) has been proposed as an approach to increase PUFA availability in ruminant products.

Ruminal BH is carried out by specific microbial consortia, with species belonging to the *Butyrivibrio* group playing a major role (Maia et al., 2007). These microorganisms convert PUFAs to stearic acid (C18:0) through a series of intermediate products, including conjugated linoleic acid (CLA) and vaccenic acid (VA). Therefore, natural compounds capable of

modifying specific steps of BH may increase the accumulation of beneficial intermediates while limiting the hydrogenation of long-chain PUFAs.

EOs are complex mixtures of bioactive compounds that have been investigated as natural modifiers of the ruminal environment (Vasta and Bessa, 2012). Recent studies have prioritised the effects of different EO constituents, including phenolic compounds and terpenoids, on ruminal microbial activity and BH (Vasta et al., 2019). However, existing data, due to differences in EO composition, concentration, and experimental conditions, remain inconsistent. For instance, thymol-rich oils have been reported to inhibit the final step of BH, reducing C18:0 formation (Makmur et al., 2023). In contrast, the impact of terpenoids, such as menthol, on the BH of fish-derived long-chain n-3 PUFAs is less explored (Ishlak et al., 2015). These findings suggest that the effects of EOs result not only from microbial inhibition but also from changes in metabolic pathways.

In the present study, we investigated the effects of thyme, peppermint, and lavender essential oils on microbial metabolism, each characterized by a distinct major bioactive constituent (thymol, menthol, and linalool, respectively). Given the unique chemical structures of these oils, we hypothesised that they would exert differential effects on the biohydrogenation of a fish oil-supplemented diet. Specifically, this study aimed to determine whether these chemotypes could act as targeted modifiers to promote ruminal vaccenic acid accumulation for CLA enrichment or to preserve n-3 PUFAs.

Materials and methods

Ethical statement, donor animals, and inoculum preparation

Rumen fluid was collected post-mortem at a commercial slaughterhouse immediately following the routine slaughter of animals for meat production. The biological material was obtained as a byproduct of commercial slaughter and no invasive or experimental procedures

were performed on live animals, thus institutional animal care and use committee approval was not required under national legislation.

Rumen fluid was collected within 10 – 15 min post-mortem from three healthy beef steers with documented health status and dietary history. Before slaughter, the animals were maintained on a standard high-forage backgrounding diet containing at least 60% forage on a dry matter (DM) basis. The animals had no prior exposure or adaptation to FO or EOs to ensure a microbial profile typical of forage-based ruminant diets.

To obtain a representative and homogenous microbial inoculum, rumen content from each steer was collected separately, filtered through four layers of cheesecloth into pre-warmed (39 °C) insulated flasks under continuous CO_2 flushing and mixed in equal volumes before being pooled for use as the inoculum in batch cultures. The pooled inoculum was then mixed with an anaerobic buffer solution prepared according to Menke and Steingass (1988) at a 1:2 (v/v) ratio under strict anaerobic conditions (pH 6.8 ± 0.05, 39 °C).

Substrate preparation and experimental design

The basal fermentation substrate used in all experimental treatments consisted of a total mixed ration (TMR; 60% alfalfa hay and 40% concentrate mixture on a DM basis) supplemented with 20 g/kg DM FO as a source of long-chain PUFAs. The chemical and fatty acid composition of the FO-supplemented TMR are presented in Table 1, whereas the fatty acid profile of the FO is provided in Table 2. The TMR contained 14.2% crude protein and 4.6% ether extract. Supplementation with 2% FO increased the concentrations of DHA (21.63 g/100 g) and EPA (8.14 g/100 g) in the diet. The FO utilised in this study had a high DHA content (26.44 g/100 g).

The experimental groups consisted of a control (FO-supplemented TMR without EO) and three EO treatments: TEO (FO-supplemented TMR + thyme [*Thymus vulgaris*] EO), LEO (FO-supplemented TMR + lavender [*Lavandula angustifolia*] EO), and PEO (FO-supplemented

TMR + peppermint [*Mentha piperita*] EO). Pure EOs were obtained from the Department of Field Crops, Faculty of Agriculture, Erciyes University. Each EO treatment was added directly to the corresponding incubation vials at a concentration of 1.5 µl/ml of buffered rumen fluid. EO composition was determined by GC/MS (Shimadzu QP2020 NX, Tokyo, Japan) using an Rtx-5MS column (Agilent, GA, USA). The oven temperature program started at 40 °C for 3 min, increased to 240 °C at a rate of 5 °C/min, then at 1 °C/min, and was finally held at 240 °C for 10 min. Volatile compounds were identified using the FFNSC3, W9N11, and NIST11 mass spectral libraries. The essential oil composition of thyme, lavender, and peppermint is shown in Table 3. Thyme EO contained mainly p-cymene (44.78%) and thymol (30.96%), lavender EO linalool (40.23%) and linalyl acetate (21.52%), and peppermint EO menthol (49.08%) and menthone (21.72%) (Table 3).

***In vitro* incubation and sampling**

Approximately 500 ± 5 mg of the respective substrate was weighed into 250 ml serum bottles, followed by the addition of 50 ml of buffered rumen fluid under a stream of CO_2. The bottles were sealed with butyl rubber stoppers and aluminium crimps. To assess the reproducibility of the *in vitro* system, incubations were performed in three independent analytical batches on different days, with five replicates per treatment in each batch (n = 5 replicates × 3 batches). Each serum bottle was considered an experimental unit.

The bottles were incubated in a shaking water bath at 39 °C for 24 h. Fermentation was terminated by placing the bottles on ice, after which the incubation fluid and residues were immediately collected for short-chain fatty acid (SCFA) and FA analyses.

Lipid extraction and fatty acid composition

The initial TMR, FO-supplemented TMR, and post-incubation residues were lyophilised using a freeze-dryer. Total lipids were extracted from the solid residue and the corresponding fluid phase according to the protocol described by Folch et al. (1957) using a chloroform-methanol (2:1, v/v) mixture. The crude lipid extract was washed with

a 7.3 g/l NaCl solution. Fatty acid methyl esters (FAME) were prepared by transesterification with 2 N methanolic KOH solution, followed by dilution in hexane and centrifugation at 4000 rpm for 10 min (Fritsche and Steinhart, 1998).

The quantitative FA composition was determined using a gas chromatograph (GC-2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector (FID) and a highly polar capillary column (HP-88, 100 m × 0.25 mm ID × 0.2 μm, Agilent Technologies, CA, USA). Individual FA CLA isomers, and medium-chain FAs were identified by comparing their retention times with external reference materials (AOAC 996.06 Standard, FAME Mix, and CLA standard). To evaluate BH, the BH rate of individual FAs was calculated after 24 h of incubation according to the following equation (expressed as g/kg of the initial FA content in the DM substrate):

Biohydrogenation rate (g/kg) = [(Initial FA content − Residual FA content)/(Initial FA content)] × 1000

The calculations were performed for oleic acid (OA), linoleic acid (LA), EPA, and DHA.

Short-chain fatty acid analysis

The relative molar proportions (%) of SCFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids) in rumen fluid were determined according to the method of Erwin et al. (1961). Briefly, 5 ml of each sample was collected and 2 drops of saturated $HgCl_2$ were added to stop fermentation. Subsequently, 1 ml of 25% metaphosphoric acid was added, the samples were mixed thoroughly, incubated for 10 min, and centrifuged at 900 × *g* for 10 min. The supernatant was passed through a 0.45 μm filter into GC vials and kept on ice until analysis. Individual SCFAs were identified by comparing their retention times with those of external standards.

Statistical analysis

Data obtained from three independent *in vitro* incubation batches were pooled and analysed using the General Linear Model (GLM) procedure of SPSS (Version 22.0; IBM Corp., Armonk, NY, USA) in a completely randomised design. The statistical model included treatment (Control vs. TEO vs. LEO vs. PEO) as a fixed effect, with the individual incubation bottle as the experimental unit (n = 15 per treatment). Homogeneity of variances and normality of residuals were verified using Levene's test and the Shapiro-Wilk test, respectively. Treatment means were compared using Tukey's *post-hoc* test. Differences were considered significant at $P < 0.05$.

Results

The effects of TEO, LEO, and PEO on the ruminal FA profile after 24 h of *in vitro* incubation are shown in Table 4. The FA profile differed significantly among treatments ($P < 0.05$). The concentration of myristic acid (C14:0) was highest in the TEO-supplemented group and lowest in the control ($P < 0.01$). Palmitic acid (C16:0) concentrations were highest in the TEO and LEO groups, and significantly lower in the PEO group ($P < 0.01$). Stearic acid (C18:0) concentrations were significantly lower in all EO-treated groups compared to the control ($P < 0.01$), indicating inhibition of the final step of BH.

The concentrations of VA (C18:1 t-11) and eicosatrienoic acid (C20:3n-6) were not significantly affected by the treatments ($P > 0.05$). OA (C18:1n-9c) concentrations were higher in the TEO and LEO groups but significantly lower in the PEO group ($P < 0.01$). LA (C18:2n-6c) reached its highest concentration in the TEO group after 24 h of incubation, whereas the lowest values were recorded in the control and PEO groups ($P < 0.01$). In contrast, gamma-linolenic acid (C18:3n-6) concentration was highest in the control and lowest in the TEO group.

The c9, t11-CLA isomer was significantly increased by TEO supplementation, reaching the highest level in this group, while the lowest concentration was observed in the PEO group. Moreover, the concentrations of EPA (C20:5n-3) and DHA (C22:6n-3) were significantly higher in all EO groups compared to the control, with the PEO group showing the greatest preservation of these long-chain n-3 FAs ($P < 0.01$).

The effects of TEO, LEO, and PEO on ruminal SCFA molar proportions after 24 h of *in vitro* incubation are shown in Table 5. Acetic acid concentration was highest in the TEO group and lowest in the control ($P < 0.05$). Propionic acid was lower in all EO groups compared to the control group ($P < 0.01$). Isobutyric acid concentration was highest in the LEO group and lowest in the PEO group ($P < 0.01$). Butyric and valeric acids were not affected by the treatments ($P > 0.05$). The acetate-to-propionate (A/P) ratio was highest in the TEO and PEO groups and lowest in the control ($P < 0.01$).

The effects of TEO, LEO, and PEO on the BH rates of long-chain FAs after 24 h of incubation are presented in Figure 1. OA BH was significantly reduced in the TEO (604.18 g/kg) and LEO (592.90 g/kg) groups than in the control (737.11 g/kg) ($P < 0.05$). LA BH was lowest in the TEO group (555.65 g/kg), differing significantly from both the control (758.32 g/kg) and PEO (750.70 g/kg) groups ($P < 0.05$). Regarding n-3 PUFAs, PEO was the most effective in reducing BH. The BH rate of EPA in the PEO group was 282.19 g/kg, representing a nearly 63% reduction compared to the control (759.19 g/kg) ($P < 0.05$). Similarly, DHA BH was lower in the PEO group (783.93 g/kg) compared to the control (947.39 g/kg) ($P < 0.05$).

Discussion

Effects on ruminal biohydrogenation

The primary objective of manipulating ruminal BH is to modify the FA profile by reducing the formation of saturated end-products. In the present study, the concentration of

palmitic acid (C16:0) was significantly lower in the PEO group than in the control ($P < 0.01$). Although palmitic acid (C16:0) is conventionally recognized for its potential to increase blood cholesterol levels, recent evidence suggests that circulating C16:0 levels are also heavily driven by *de novo* lipogenesis from carbohydrate intake rather than dietary fat alone (Keys et al., 1965; Givens, 2022). The lower concentrations of C14:0 and C16:0 in the PEO group suggest that menthol-rich EOs may influence the metabolic pathways involved in *de novo* FA synthesis or the early stages of FA saturation (Ishlak et al., 2015). Unlike TEO and LEO, which increased concentrations of these SFAs, PEO appears to be more effective candidate for reducing the hypercholesterolemic risk associated with dairy fat consumption (Givens, 2022).

In the present study, the concentration of stearic acid (C18:0), the final end-product of C18-series BH, was significantly lower in all EO groups compared to the control ($P < 0.01$). This finding indicates inhibition of the final step of BH (Vasta et al., 2019). The accumulation of intermediates, particularly *c9,t11*-CLA, together with the lower C18:0 concentration, suggests that EOs, especially TEO, inhibited the development of microorganisms from the *Butyrivibrio* group, including *Butyrivibrio proteoclasticus* (Maia et al., 2007).

The significant increase in the concentration of *c9,t11*-CLA and LA (C18:2 n-6) in the TEO group confirms the effective accumulation of vaccenic acid in the rumen. Reduced conversion of VA to C18:0 may increase the flow of VA to the lower digestive tract (Frutos et al., 2020). As VA is the main precursor of endogenous CLA synthesis in the mammary gland and muscle catalysed by delta-9 desaturase, its accumulation is considered beneficial (Griinari et al., 2000; Piperova et al., 2002). The higher CLA and lower C18:0 concentrations in the TEO group are consistent with reports that thymol disrupts the membrane integrity of terminal BH-inducing bacteria (Cobellis et al., 2016). Similarly, the lower LA BH observed in the TEO group (555.65 g/kg vs. 758.32 g/kg in the control) supports the inhibitory effect of thymol-rich EOs on *Butyrivibrio* spp. (Figure 1). The effects of LEO on BH, may be related to its dominant

constituents, linalool and linalyl acetate, which show intermediate antimicrobial activity against ruminal microorganisms (Benchaar et al., 2008). These differences in chemical composition may explain why TEO promoted the greatest CLA accumulation, while PEO and LEO differed in their ability to preserve unsaturated intermediates (Benchaar et al., 2008; Lourenço et al., 2010).

An interesting finding was the greater preservation of long-chain n-3 FAs in the PEO group than in the control and other EO groups ($P < 0.01$). Although the BH pathways of 20- and 22-carbon PUFAs have not been fully elucidated (Toral et al., 2018), the present data suggest that menthol- and menthone-rich PEO may reduce the microbial conversion of these FAs (Calsamiglia et al., 2007). The decrease in EPA BH to 282.19 g/kg in the PEO group (a 63% decrease compared to the control group) supports this interpretation. Similarly, DHA BH was lowest in the PEO group (783.93 g/kg) (Figure 1). This effect could be related to the selective antimicrobial activity of terpenoids against specific microbial consortia, such as certain protozoa or specialised cellulolytic clades, that participate in the BH of n-3 fatty acids (Patra and Saxena, 2010; Toral et al., 2018). By reducing the BH of EPA and DHA, PEO could potentially increase their flow to the duodenum, thereby increasing their concentration in ruminant-derived products (Wachira et al., 2000; Chikunya et al., 2004).

Effects on rumen short chain fatty acids

Changes in SCFA proportions reflect alterations in ruminal fermentation. Supplementation with TEO significantly increased the A/P ratio ($P < 0.01$), altered hydrogen metabolism during fermentation (Benchaar et al., 2008). Considering that BH is an important hydrogen-utilising process in the rumen, its suppression by EOs may alter hydrogen use and contribute to maintaining ruminal redox balance (Ungerfeld, 2020).

The increase in A/P ratio, a major precursor of milk fat synthesis, combined with the reduction in propionate, suggests that TEO shifts fermentation towards cellulolytic pathways

leading to the production of acetate rather than propionate (Cobellis et al., 2016). Maintaining total SCFA concentrations indicates that the applied EO dose acted as selective metabolic modulators rather than a broad-spectrum inhibitor (Castillejos et al., 2006). While some studies have reported inconsistent effects on the A/P ratio depending on dosage, the present findings demonstrate that the EO dose used selectively modified ruminal fermentation without inducing general microbial toxicity (Spanghero et al., 2008; Vasta et al., 2019).

Conclusions

The results of the current study demonstrate that thyme, lavender, and peppermint essential oils differ in their effects on ruminal biohydrogenation. Supplementation with dietary essential oils significantly inhibited the final reductive step of biohydrogenation *in vitro*, resulting in lower stearic acid (C18:0) concentrations. Among the tested essential oils, thyme oil application resulted in the highest c9,t11-CLA concentration, whereas peppermint (*Mentha piperita*; PEO) essential oil was associated with the highest eicosapentaenoic acid and docosahexaenoic acid concentrations, and the lowest levels of hypercholesterolemic saturated fatty acids, such as C16:0.

These findings suggest that the effects of essential oils on ruminal biohydrogenation depend on their chemical composition. The high potential of thyme (*Thymus vulgaris*; TEO) to increase conjugated linoleic acid (CLA) concentration and of peppermint (to preserve beneficial n-3 fatty acids may contribute to the manufacture of ruminant products with an improved fatty acids profile. Future *in vivo* research is required to validate the stability of these effects and to precisely identify the microbial taxa involved. Studies evaluating different essential oils doses and combinations may contribute to the development of nutritional strategies to improve the fatty acid profile of milk and meat without adversely affecting ruminal fermentation.

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Conflict of interest

The Authors declare that there is no conflict of interest.

REFERENCES

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, 209–228, https://doi.org/10.1016/j.anifeedsci.2007.04.014

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, https://doi.org/10.3168/jds.2006-644

Castillejos L., Calsamiglia S., Ferret A., 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. J. Dairy Sci. 89, 2649–2658, https://doi.org/10.3168/jds.S0022-0302(06)72341-4

Chikunya S., Demirel G., Enser M., Wood J.D., Wilkinson R.G., Sinclair L.A., 2004. Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. Br. J. Nutr. 91, 539–550, https://doi.org/10.1079/BJN20031078

Cobellis G., Trabalza-Marinucci M., Yu Z., 2016. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Sci. Total Environ. 545, 556–568, https://doi.org/10.1016/j.scitotenv.2015.12.103

Erwin E., Marco G., Emery E., 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44, 1768–1771, https://doi.org/10.3168/jds.S0022-0302(61)89956-6

Folch J., Lees M., Sloane-Stanley G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509, https://doi.org/10.1016/S0021-9258(18)64849-5

Fritsche J., Steinhart H., 1998. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z. Lebensm. Unters. Forsch. A 206, 77–82, https://doi.org/10.1007/s002170050218

Frutos P., Hervás G., Natalello A., Luciano G., Fondevila M., Priolo A., Toral P.G., 2020. Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles. Anim. Feed Sci. Technol. 269, 114623, https://doi.org/10.1016/j.anifeedsci.2020.114623

Givens D.I., 2022. Saturated fats, dairy foods and cardiovascular health: No longer a curious paradox? Nutr. Bull. 47, 407–422, https://doi.org/10.1111/nbu.12585

Givens D.I., Shingfield K.J., 2004. Foods derived from animals: the impact of animal nutrition on their nutritive value and ability to sustain long-term health. Nutr. Bull. 29, 325–332, https://doi.org/10.1111/j.1467-3010.2004.00444.x

Griinari J.M., Corl B.A., Lacy S.H., Chouinard P.Y., Nurmela K.V., Bauman D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta 9-desaturase. J. Nutr. 130, 2285–2291, https://doi.org/10.1093/jn/130.9.2285

Ishlak M., 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, https://doi.org/10.1016/j.anifeedsci.2015.05.023

Kepler C.R., Tove S.B., 1967. Biohydrogenation of unsaturated fatty acids. 3. Purification and properties of a linoleate delta-12-cis, delta-11-trans-isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem. 242, 5686–5692, https://doi.org/10.1016/S0021-9258(18)99355-5

Keys A., Anderson J.T., Grande F., 1965. Serum cholesterol response to changes in the diet: III. Differences among individuals. Metabolism 14, 766–775, https://doi.org/10.1016/0026-0495(65)90003-X

Lourenço M., Ramos-Morales E., Wallace R.J., 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal 4, 1008–1023, https://doi.org/10.1017/S175173111000042X

Maia M., Chaudhary L., Figueres L., Wallace R.J., 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek 91, 303–314, https://doi.org/10.1007/s10482-006-9118-2

Makmur M., Yanza Y.R., Fitri A., Syarifuddin, Ridwan R., Jayanegara A., 2023. Effects of essential oils and their derivatives on rumen fermentation characteristics and PUFA biohydrogenation: A meta-analysis of *in vitro* studies. Vet. Integr. Sci. 21, 925–944, https://doi.org/10.12982/VIS.2023.067

Menke K.H., Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28, 7–55

Patra A.K., Saxena J., 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry 71, 1198–1222, https://doi.org/10.1016/j.phytochem.2010.05.010

Piperova L.S., Sampugna J., Teter B.B., Kalscheur K.F., Yurawecz M.P., Ku Y., Morehouse K.M., Erdman R.A., 2002. Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of cis-9-containing CLA in lactating dairy cows. J. Nutr. 132, 1235–1241, https://doi.org/10.1093/jn/132.6.1235

Spanghero M., Zanfi C., Fabbro E., Scicutella N., Camellini C., 2008. Effects of a blend of essential oils on some end products of in vitro rumen fermentation. Anim. Feed Sci. Technol. 145, 364–374, https://doi.org/10.1016/j.anifeedsci.2007.05.048

Toral P.G., Monahan F.J., Hervás G., Frutos P., Moloney A.P., 2018. Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities. Animal 12, s272–s281, https://doi.org/10.1017/S1751731118001994

Ungerfeld E.M., 2020. Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. Front. Microbiol. 11, 589, https://doi.org/10.3389/fmicb.2020.00589

Vasta V., Bessa R.J.B., 2012. Manipulating ruminal biohydrogenation by the use of plants bioactive compounds. In: A. Patra (Editor). Dietary Phytochemicals and Microbes. Springer, Dordrecht (The Netherlands), pp. 139–168, https://doi.org/10.1007/978-94-007-3926-0_9

Vasta V., Daghio M., Cappucci A., Buccioni A., Serra A., Viti C., Mele M., 2019. Invited review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches. J. Dairy Sci. 102, 3781–3804, https://doi.org/10.3168/jds.2018-14985

Wachira A.M., Sinclair L.A., Wilkinson R.G., Hallett K., Enser M., Wood J.D., 2000. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. J. Agric. Sci. 135, 419–428, https://doi.org/10.1017/S0021859699008370

Table 1. Feed ingredients, chemical, and fatty acid composition of the experimental diet

Ingredients	g/kg DM	Fatty acids	g/100g in FAME
Alfalfa hay	600	(C14:0) Myristic Acid	5.19
Barley grain	174	(C16:0) Palmitic Acid	19.77
Corn grain	81	(C16:1) Palmitoleic Acid	5.04
Sunflower meal	45	(C17:0) Heptadecanoic Acid	1.58
Soybean meal	15	(C18:0) Stearic Acid	4.43
Corn DDGS	26	(C18:1n9c) Oleic Acid	18.39
Molasses	4	(C18:2n6c) Linoleic Acid	7.86
Marble	9	(C18:3n6) y-Linolenic Acid	1.49
Wheat bran	21	(C20:1n9) Eicosenoic Acid	3.43
Vit-Min Premix*	5	(C18:3n3) a-Linolenic Acid	0.81
Fish oil	20	(C20:5n3) Eicosapentaenoic Acid	8.14
		(C22:6n3) Docosahexaenoic Acid	21.63
Chemical Composition		Other Fatty acids	2.24
DM, %	88.6		
CP, % DM	14.2		
EE, % DM	4.6		
NDF, % DM	32.6		
ADF, % DM	18.4		
ME, Mcal/kg DM	2.6		

FAME – fatty acid methyl esters; DM – dry matter, CP – crude protein, E.E. – ether extract, NDF – neutral detergent fiber, ADF – acid detergent fiber, ME – metabolizable energy; * Contains in per kilogram: IU: vitamin A 5000, vitamin D3 600; mg: Fe 50, Mn 30, Co 0.18, Se 0.15, Zn 40, I 0.8, Cu 10

Table 2. Fatty acid composition of fish oil used in the substrate diet

Fatty acids	g/100 g in FAME
(C14:0) Myristic Acid	6.17
(C16:0) Palmitic Acid	21.08
(C16:1) Palmitoleic Acid	6.18
(C18:0) Stearic Acid	4.82
(C18:1n9c) Oleic Acid	19.89
(C18:2n6c) Linoleic Acid	2.65
(C20:5n3) Eicosapentaenoic Acid	9.95
(C22:6n3) Docosahexaenoic Acid	26.44
Others*	2.82

*Sum of fatty acids detected in amounts less than 1%. FAME – fatty acid methyl esters

Tablo 3. Thyme (*Thymus vulgaris* L.), Lavander (*Lavandula angustifolia* Mill.) and Peppermint (*Mentha piperita* L.) essential oil composition

Compound (Thyme)	%	Compound (Lavander)	%	Compound (Peppermint)	%
Alpha-Pinene	1.28	1,8-Cineole	5.12	1,8-Cineole	5.8
1-Octen-3-Ol	1.45	Cis-Linalool Oxide	1.07	Trans-Sabinene Hydrate	2.2
Para-Cymene	44.78	Linalool	40.23	Menthone	21.72
Gamma-Terpinene	1.03	Camphor	8.37	Neo-Menthol	6.8
Linalool	2.53	Borneol	3.49	Menthol	49.08
Borneol	1.2	Alpha Terpineol	4.49	Terpinene-4-Ol	1.28
Thymol	30.96	Linalyl Acetate	21.52	Menthyl Acetate	3.62
Carvacrol	3.97	Lavandulyl Acetate	2.6	Others*	9.5
Others*	12.8	Geranyl Acetate	1.74		
		Others*	15.86		

*Sum of components detected in amounts less than 1%

Table 4. The effects of essential oil supplementation to fish oil-supplemented diet on ruminal fatty acid composition after 24 h incubation

Fatty acids g/100 g in FAME	**CON**	**TEO**	**LEO**	**PEO**	**SEM**	***P*-value**
C14:0 Myristic Acid	3.56c	6.11a	5.16ab	4.43bc	0.30	0.000
C16:0 Palmitic Acid	15.50b	18.37a	17.89a	13.07c	0.67	0.000
C16:1 Palmitoleic Acid	2.99b	3.92a	3.96a	2.79b	0.20	0.031
C18:0 Stearic Acid	40.61a	19.45b	22.81b	24.81b	2.56	0.000
C18:1 *t-11* Vaccenic Acid	3.01	3.91	3.90	2.74	0.21	0.064
C18:1 *n9c* Oleic Acid	10.67b	15.02a	15.05a	9.12b	0.82	0.000
C18:2 *n6c* Linoleic Acid	4.20c	7.21a	5.92b	4.17c	0.41	0.000
C18:3 *n6* Linolenic Acid	10.17a	5.42c	7.18bc	7.61b	0.56	0.002
CLA *c-9 t-11*	1.44bc	2.15a	1.76ab	1.25c	0.12	0.008
C20:3 *n6* Eicosatrienoic Acid	4.71	3.59	4.58	5.06	0.63	0.000
C20:5 *n3* Eicosapentaenoic Acid	4.33c	6.40b	6.58b	12.40a	0.92	0.000
C22:6 *n3* DocosahexaenoicAcid	2.50c	6.44b	4.48bc	9.92a	0.87	0.000

FAME – fatty acid methyl esters; CON – control (diet supplemented with 2% fish oil), TEO – control + thyme essential oil (1.5 µl/ml rumen fluid), LEO – control + lavender essential oil (1.5 µl/ml rumen fluid), PEO – control + peppermint essential oil (1.5 µl/ml rumen fluid); SEM – standard error of means; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 5. The effects of essential oil supplementation to fish oil-supplemented diet on ruminal volatile fatty acid (VFA) molar proportions after 24 h incubation

VFA	**CON**	**TEO**	**LEO**	**PEO**	**SEM**	***P*-value**
Acetic acid	53.58^{b}	55.96^{a}	54.95^{ab}	54.55^{ab}	0.34	0.052
Propionic acid	20.14^{a}	17.21^{c}	17.05^{c}	18.48^{b}	0.39	0.000
Isobutyric acid	1.85^{b}	1.89^{b}	2.91^{a}	1.30^{c}	0.18	0.000
Butyric acid	16.74	17.13	17.54	17.69	0.16	0.140
Isovaleric acid	4.41^{ab}	4.47^{ab}	3.98^{b}	4.92^{a}	0.12	0.023
Valeric acid	3.29	3.34	3.57	3.06	0.10	0.366
A/P	2.66^{c}	3.25^{a}	3.22^{a}	2.95^{b}	0.08	0.000

CON – control (diet supplemented with 2% fish oil), TEO – control + thyme essential oil (1.5 µl/ml rumen fluid), LEO – control + lavender essential oil (1.5 µl/ml rumen fluid), PEO – control + peppermint essential oil (1.5 µl/ml rumen fluid); A/P – acetate/ propionate ratio; SEM – standard error of means; ^{abc} - means within a row with different superscripts are significantly different at $P < 0.05$

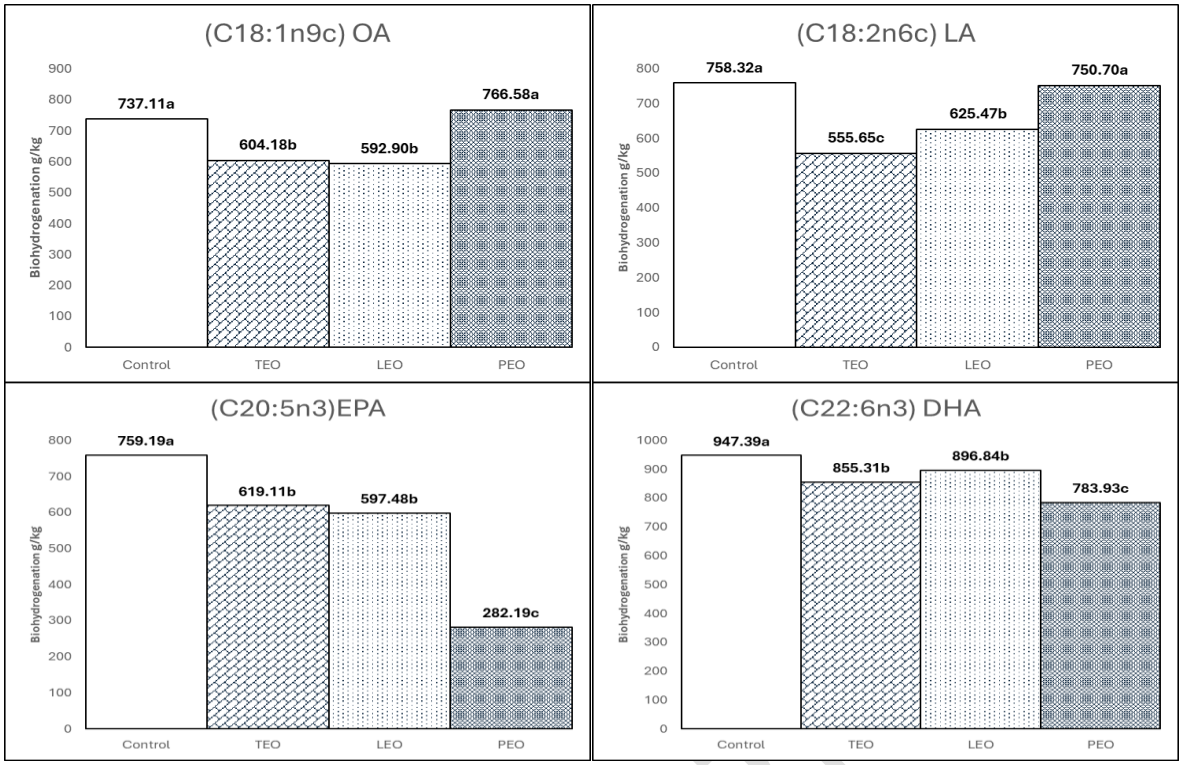

Figure 1. Effects of adding thyme, lavender, and peppermint essential oils to a fish oil diet on the biohydrogenation rate of oleic (OA), linoleic (LA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) fatty acids after 24-hour *in vitro* incubation; different letters (a, b, c) within the same fatty acid category indicate significant differences between treatments ($P < 0.05$)