Received: 25 November 2019 | Revised: 3 March 2020 | Accepted: 21 March 2020

DOI: 10.1111/jpn.13367

REVIEW

Journal of Animal Physiology and Animal Nutrition | WILEY

The effects of dietary medium-chain fatty acids on ruminal methanogenesis and fermentation in vitro and in vivo: A meta-analysis

Yulianri Rizki Yanza[1,2] | Małgorzata Szumacher-Strabel[1] | Anuraga Jayanegara[2,3] | Andre Meiditama Kasenta[2,4] | Min Gao[1] | Haihao Huang[1] | Amlan Kumar Patra[5] | Ewelina Warzych[6] | Adam Cieślak[1]

[1]Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poznań, Poland

[2]Animal Feed and Nutrition Modelling Research Group (AFENUE), Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia

[3]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia

[4]Livestock Production Program, Polytechnic Agriculture and Animal Science (MAPENA), Tuban, Indonesia

[5]Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, India

[6]Department of Genetics and Animal Breeding, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poznań, Poland

Correspondence
Adam Cieślak, Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poznań, Poland.
Email: adam.cieslak@up.poznan.pl

Funding information
Narodowe Centrum Nauki, Grant/Award Number: UMO-2016/23/B/NZ9/03427; Polish National Agency for Academic Exchange (NAWA), Grant/Award Number: Ignacy Lukasiewicz PhD scholarship 015/IL/1617; Faculty of Veterinary Medicine and Animal Science Poznan University of Life Sciences, Grant/Award Number: 506.533.04; Ministerstwo Nauki i Szkolnictwa Wyższego, Grant/Award Number: 005/RID/2018/19

Abstract

The efficacy of methane (CH_4) suppression using medium-chain fatty acids (MCFA) remains inconclusive, despite a number of studies on this topic are available. We thus carried out a meta-analysis to integrate the published data on different concentrations and types of MCFA such as lauric acid and myristic acid, which investigated ruminal methanogenesis and fermentation in in vitro and in vivo experiments. In vitro MCFA sources were classified either as pure MCFA (lauric acid, myristic acid and their combinations) or as natural MCFA-rich oils (canola oil enriched with lauric acids, coconut oil, krabok oil and palm kernel oil). The MCFA sources used in the in vivo studies were coconut oil, lauric acid, myristic acid and the combination of lauric and myristic acids. A total of 41 studies (20 in vitro and 21 in vivo studies) were compiled in our database, which included the data on CH_4 emission, digestibility, ruminal fermentation products and microbial populations. The results showed that the amount of CH_4 production per unit of digested organic matter decreased linearly under in vitro conditions ($p < .01$) and tended to decrease quadratically under in vivo conditions ($p < .07$) with increasing doses of MCFA. Populations of protozoa ($p < .01$) in both in vitro and in vivo responded negatively in a linear manner, whereas *Archaea* population diminished quadratically ($p = .04$) only in the in vitro conditions with increasing doses of MCFA. Increasing dietary MCFA concentrations also reduced the fibre digestibility linearly ($p < .05$) in both in vitro and in vivo conditions. CH_4 production for different sources of MCFA decreased in following order: coconut oil > lauric acid > myristic acid > mixed lauric and myristic acids > palm kernel oil > canola oil enriched with lauric acids > krabok oil. It can be concluded that the effect of MCFA on ruminal methanogenesis depends on the amount and type of MCFA.

KEYWORDS
medium-chain fatty acid, methanogenesis, ruminal fermentation, ruminants

1 | INTRODUCTION

Ruminants have been implicated as significant contributors to global emissions of anthropogenic greenhouse gases, including methane (CH_4) that has a 100-year global warming potential of 28 times greater than that of carbon dioxide (Myhre et al., 2013). The contribution of enteric CH_4 emission from the ruminants has been estimated at about 17% of the global greenhouse gas outputs, producing approximately 47% of global livestock-sector greenhouse gas emissions (FAO, 2013; Knapp, Laur, Vadas, Weiss, & Tricarico, 2014). Methane emission also involves feed energy losses ranging from 2% to 15%, depending on the diet type, level of intake and feed composition (Giger-Reverdin & Sauvant, 2000; Johnson & Johnson, 1995). Reducing the enteric CH_4 production from ruminants will not only substantially decrease greenhouse gas emissions, but also will reduce dietary energy losses. Many dietary and animal management strategies have been proposed to reduce CH_4 emissions from ruminants, especially from dairy cattle (Knapp et al., 2014; Patra, 2016). Effective methods of mitigating CH_4 emissions from ruminants with less negative effects on ruminal fermentation are needed for the sustainable adoption of the strategies (Patra, Park, Kim, & Yu, 2017).

Methane is produced by *Archaea* in the rumen, mainly utilizing H_2 and CO_2 (Patra et al., 2017). Supplementation with chemical additives can decrease *Archaea* and bacterial populations, leading to lowered CH_4 emission from the rumen (Patra et al., 2017; Zhang et al., 2018). However, such interventions may also decrease nutrient digestibility and affect animal products with their residues (Patra et al., 2017). It is thus important to explore natural and biotechnological interventions to alleviate this problem with the minimum side effects (Bošnjak, Prpić, Ugarković, Konjačić, & Vnučec, 2018; Varadyova, Certik, & Jalc, 2018).

Supplementation of dietary fat has been investigated in ruminant nutrition and mitigation of CH_4 production for decades (Blaxter & Czerkawski, 1966; Patra, 2014; Szczechowiak et al., 2018). Generally, pure sources of medium-chain fatty acids (MCFA), including lauric acid (LA; C12:0), myristic acid (MA; C14:0) and their combinations, showed stronger CH_4 mitigation effects than long-chain saturated fatty acids due to their greater inhibitory effects on methanogens (Patra, 2013; Soliva, Meile, Cieślak, Kreuzer, & Machmüller, 2004; Van Zijderveld et al., 2011). Coconut oil, krabok oil and palm kernel oil as rich sources of MCFA have also been investigated for their responses on CH_4 production, feed digestibility, bacterial populations, ruminal fermentation, milk production and blood parameters, but the results are not always conclusive (Kang, Wanapat, & Viennasay, 2016; Klop, Dijkstra, Dieho, Hendriks, & Bannink, 2017; Machmüller, Ossowski, Wanner, & Kreuzer, 1998; Yabuuchi, Matsushita, Otsuka, Fukamachi, & Kobayashi, 2006). Previous studies had tested the MCFA effect on CH_4 production both in vitro and in vivo. The contrasted results between these two experimental conditions might be inevitable. However, there is potential to predict and estimate in vivo response of methane production from results obtained using in vitro methods (Bhatta et al., 2007; Jayanegara, Leiber, & Kreuzer, 2012).

A meta-analysis approach, which increases the power of the statistical analysis (Jayanegara et al., 2012; Patra, 2009), can help to clarify the effects of MCFA sources on ruminal methanogenesis. We hypothesized that the effect of different sources of MCFA on ruminal methanogenesis and fermentation can be better understandable through meta-analysis approaches. The aim of the present study was to perform a meta-analysis of published in vitro and in vivo experiments to investigate the effects of various MCFA sources (mainly LA and MA) on methanogenesis, as well as on ruminal fermentation parameters, digestibility and microbial populations.

2 | MATERIALS AND METHODS

2.1 | Database development

The search engines of journal collections such as Web of Science, Scopus and Science Direct were used to collect papers on the relationship between MCFA sources and CH_4 production (Figure 1). The following criteria were used for the inclusion of articles into the database: (a) they described in vitro or in vivo studies in ruminants; (b) they involved adding MCFA to basal feeds; (c) CH_4 emission was directly measured (calculating CH_4 emission data were excluded); and (d) the articles were published in English. The selected published in vitro and in vivo studies that reported the effects of MCFA (i.e. LA and MA) on ruminal CH_4 production, volatile fatty acid (VFA) concentration, digestibility and microbial population were compiled in a database (Tables 1 and 2). The MCFA were used from synthetic sources of single free fatty acids, such as LA, MA, and mixed lauric and myristic acids (LMA), as well as natural sources such as coconut oil (CO), krabok oil (KO), canola oil enriched with lauric acid (CanO) and palm kernel oil (PKO). The levels of such addition and their sources were noted in the database.

The final database contained 20 in vitro studies with 154 treatment means (Table 1) and 21 in vivo studies with 75 treatment means (Table 2). The number of in vitro and in vivo observations across variables was not constant, and a descriptive statistic of the data is presented in Table 3. Various in vitro techniques were noted, such as the rumen simulation technique (RUSITEC; Czerkawski & Breckenridge, 1977), the Hohenheim gas test incubation (HGT; Menke et al., 1979), consecutive batch culture (CBC), glass bottle incubation (GBI), the gas production technique (GPT) and the glass vessel incubation (GVI). Most in vitro studies used basal diets such as hay, grass, alfalfa, maize, grains and concentrates as the main substrates (Table 1). In the in vivo studies, the animals which received the MCFA treatments were dairy cattle, steers, sheep and lambs. These animals received the basal diets consisted mostly of hay, alfalfa silage, maize silage, grains, soybean meal and concentrates.

We expressed the MCFA concentrations as g/kg dry matter (DM) of the substrate. Measurements expressed in other units (such as mg/mL, % v/v, or % w/v) in the studies were converted to g/kg DM substrate using the information available in the papers. Fatty acid sources, such as LA and MA in various forms, LMA,

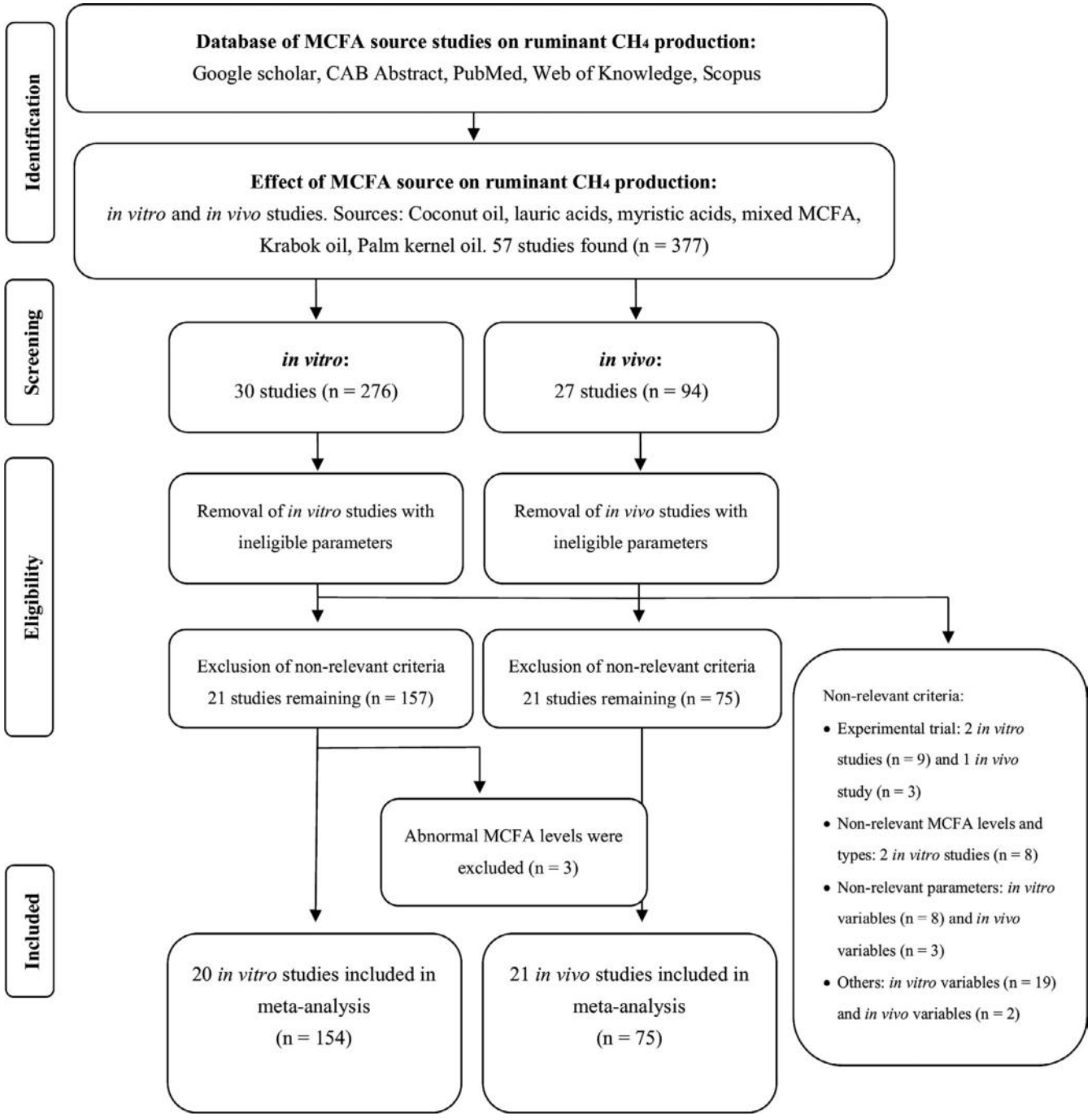

FIGURE 1 Flow diagram for selection of the studies in the meta-analysis to investigate the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis in the in vitro and in vivo studies

CO, KO, CanO and PKO, were converted to g MCFA/kg DM on the basis of their total LA and MA contents. The concentration of CH_4 in the in vitro studies, as determined by gas chromatography (GC), was included as the primary CH_4 emission parameter. In the in vivo studies, the CH_4 emission was determined by the SF6 tracer gas methods and using respiration chambers. The CH_4 emission in millilitres was converted to litres of CH_4 gas produced per kilogram of substrate (i.e. L/kg DM of substrate) for the in vitro studies and L/kg DM intake for the in vivo studies, as well as the L/kg digested organic matter (OM) for the both in vitro and in vivo studies. Furthermore, CH_4 production for all the studies expressed per unit of OM digested was also converted to a percentage change relative to the intercept values. Ruminal fermentation characteristics (NH_3 and total VFA concentration), degradability of DM, OM, crude protein (CP), and neutral detergent fibre (NDF) and microbial populations (bacteria, protozoa and *Archaea*) in the various studies were converted into the same units of measurement to allow direct analysis within a specified parameter.

TABLE 1 In vitro experiments included in the meta-analysis of the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis

Exp. No.	Reference	Method	Diet	Source	MCFA level, g/kg DM
1	Dong et al. (1997)	RUSITEC	Grass	CO	0–63.8
2	Machmüller et al. (1998)	RUSITEC	Concentrates	CO	0–25.2
3	Dohme et al. (1999)	RUSITEC	Maize silage, concentrate	CO	0–35.1
4	Dohme et al. (2000)	RUSITEC	Maize silage, concentrates	CanO, PKO, CO	0.8–47.7
5	Dohme et al. (2001)	RUSITEC	Maize silage, concentrate	LA, MA	0–45
6	Machmüller, Dohme, Soliva, Wanner, and Kreuzer (2001)	RUSITEC	Hay, corn silage, concentrates	CO	0–33.1
7	Machmüller et al. (2001)	RUSITEC	Hay, corn silage, concentrates	LA	0–66
8	Machmüller, Soliva, and Kreuzer (2002)	RUSITEC	Hay, corn silage, concentrates	LA	0–50
9	Ajisaka et al. (2002)	GVI	Glucose, cellobiose	LA	0–41.7
10	Soliva et al. (2003)	HGT	Not specified	LA, MA, LMA	0–27
11	Soliva et al. (2004)	RUSITEC	Grass hay, concentrate	LA, MA, LMA	0–48.5
12	Yabuuchi et al. (2006)	GBI	Corn grains	PKO, CO	0–73.5
13	Cieślak et al. (2006)	RUSITEC	Hay; wheat meal	CO	0–32.5
14	Božic et al. (2009)	CBC	Alfalfa	LA	0–22.5
15	Klevenhusen et al. (2009)	RUSITEC	Maize, wheat; hay	LA	0–36.5
16	Staerfl, Kreuzer, and Soliva (2010)	HGT	Maize silage, concentrate	LA, MA	0–50
17	O'brien, Navarro-Villa, Purcell, Boland, and O'Kiely (2014)	GPT	Ryegrass, grass silage, barley grains	LA	0–99
18	Kim et al. (2014)	GVI	Alfalfa; concentrate	LA	0–69.9
19	Kang et al. (2016)	GBI	Cassava chips, rice bran; coconut and palm meals	KO, CO	0–17.7
20	Kang et al. (2017)	GBI	Rice straw, cassava	KO	0–44.2

Abbreviations: CanO, canola oil enriched with lauric acid; CBC, batch culture; DM, dry matter; GBI, glass bottle incubation; GPT, gas production technique; GVI, glass vessel incubation; HGT, Hohenheim gas test; KO, krabok oil; LA, lauric acid; LMA, mixed lauric and myristic acids; MA, myristic acid; MCFA, medium-chain fatty acids ($C_{12:0}$ and $C_{14:0}$) CO, coconut oil; PKO, palm kernel oil; RUSITEC, rumen simulation technique.

2.2 | Statistical analysis

The collected data were statistically analysed using a mixed-model meta-analysis approach (Patra, 2013; Sauvant, Schmidely, Daudin, & St-Pierre, 2008; St-Pierre, 2001). The analysis employed the PROC MIXED procedure of SAS 9.4 software (University Ed., online). The studies were taken as the random effects, while the concentrations of MCFA supplementations were taken as the fixed effects. Statistical models were applied for both the continuous and discrete variables. The continuous predictor variable consisted of the concentrations of MCFA supplementation. The statistical model used was (Equation 1):

$$Y_{ij} = B_0 + B_1 X_{ij} + B_2 X_{ij}^2 + s_i + b_i X^{ij} + e_{ij} \quad (1)$$

where Y_{ij} = dependent variable; B_0 = overall intercept across all studies (fixed effect); B_1 = linear regression coefficient of Y on X (fixed effect); B_2 = quadratic regression coefficient of Y on X (fixed effect); X_{ij} = value of the continuous predictor variable (MCFA concentration); s_i = value of random effect of study *i*; b_i = random effect of study on the regression coefficient of Y on X in study *i*; and e_{ij} = the unexplained residual error. The CLASS statement was declared on the basis of MCFA levels and the study variable, since they did not contain any quantitative information. The RANDOM statement was declared on the basis of different studies, due to varying concentrations of dietary MCFA used in the in vitro and in vivo approaches. These models were used by weighting the number of replicates in the studies, as done by Jayanegara, Wina, and Takahashi (2014). The model was considered significant at $p \leq .05$ or tended to be significant at $0.05 < p \leq .10$.

To graphically present the data arising from the multidimensional space of studies in a two-dimensional space, with the predicted Y values on the regression line with the residuals, the responses variable (Y) was adjusted to take into account of the random effect of the studies (Jayanegara et al., 2014; St-Pierre, 2001). However, changes in CH_4/digested OM were calculated by subtracting the intercept of the regression equation (i.e. the value at dietary MCFA equal to 0) from each CH_4/digested OM value and were expressed as a proportion of the intercept following the method of Jayanegara et al. (2012). Thus, the typical experimental

TABLE 2 In vivo experiments included in the meta-analysis of the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis

Exp. No.	Reference	Animal	Feeding ration	Source	MCFA level, g/kg DM
1	Machmüller and Kreuzer (1999)	Sheep	Hay, concentrate	CO	0–36.2
2	Machmüller, Ossowski, and Kreuzer (2000)	Lamb	Hay, concentrate, maize silage	CO	0–23.4
3	Machmüller et al. (2003)a	Lamb	Hay, concentrate	MA	0–49
4	Machmüller et al. (2003)b	Lamb	Maize silage, concentrate	CO	0–35
5	Lovett et al. (2003)	Dairy cattle	Rolled barley, soybean meal	CO	0–75.4
6	Jordan, Lovett, Hawkins, Lovett, Hawkins, Callan, and O'Mara (2006)	Dairy cattle	Grass, barley, soybean meal	CO	0–33.9
7	Jordan, Lovett, Monahan, et al. (2006)	Dairy cattle	Barley, soybean meal	CO	0–81.3
8	Sauvant et al. (2008)	Dairy cattle	Cassava chips, soybean meal, coconut meal	CO	0–19.5
9	Crompton, Mills, and Reynolds (2010)	Dairy cattle	Maize, grass silage, cereals	CO	0–22.8
10	Hristov et al. (2009)	Dairy cattle	Alfalfa, barley, corn	LA, CO	0–14.3
11	Ding et al. (2012)	Female yaks	Grass, oat hay	CO	0–78
12	Kongmun, Wanapat, Nontaso, Nishida, and Angthong (2009)	Steer	Rice straw, concentrate	CO	0–45.5
13	Pilajun, Wanapat, Wachirapakorn, and Navanukroaw (2010)	Steer	Rice straw, concentrate	CO	7.1–28.2
14	Van Zijderveld et al. (2011)	Dairy cattle	Maize, dry beet pulp, barley	LA, MA	0–16
15	Liu, Vaddella, and Zhou (2011)	Lamb	Barley silage, concentrate	CO	0–14.8
16	Hollmann et al. (2012)	Dairy cattle	Alfalfa, corn, grass	CO	0–19.9
17	Ding et al. (2012)	Sheep	Oat hay	CO	0–11.6
18	Hollmann, Powers, Fogiel, Liesman, and Beede (2013)	Dairy cattle	Alfalfa, corn, soy hull	CO	0–24.1
19	Nguyen and Hegarty (2017)	Dairy cattle	Oaten, alfalfa	CO	0–26.5
20	Klop et al. (2017)	Dairy cattle	Corn, soybean meal	LA	0–63.7
21	Suryani, Zain, Ningrat, and Jamarun (2017)	Steer	Palm oil frond	CO	0–13.9

Abbreviations: DM, dry matter; LA, lauric acid; MA, myristic acid LMA, Mixed lauric and myristic acids; MCFA, medium-chain fatty acids ($C_{12:0}$ and $C_{14:0}$) CO, coconut oil.

approaches (in vitro or in vivo) were considered random factors, which make the suitability of the in vitro values predicting the in vivo CH_4 changes/digested OM.

The effectiveness across sources of MCFA was compared using the following statistical model for the discrete predictor variables (MCFA sources; (Equation 2):

$$Y_{ij} = \mu + s_i + \tau_j + s\tau_{ij} + e_{ij} \quad (2)$$

where Y_{ij} = dependent variable, μ = overall mean, s_i = random effect of the i-th study, τ_j = fixed effect of the j-th level of factor τ, $s\tau_{ij}$ = random interaction between the i-th study and the j-th level of factor τ, and e_{ij} = the unexplained residual error, as described by Jayanegara et al. (2014). The different MCFA sources were taken as the fixed effect. When a variable showed a significant difference at $p \leq .05$ or a tendency at $0.05 < p \leq .10$ across the various MCFA sources, least square means and Tukey's post hoc test was used to compare the difference among the means.

3 | RESULTS

3.1 | In vitro experiments

The addition of MCFA in in vitro studies had no effect on the degradability of OM or CP, but linearly decreased DM degradability ($p = .02$; $R^2 = 0.32$) and NDF degradability ($p < .01$; $R^2 = 0.71$; Table 4). CH_4 emission, both expressed as volume (Litre; $p = .03$; $R^2 = 0.62$) and as L/g DM substrate ($p = < 0.01$; $R^2 = 0.72$), decreased linearly with increasing doses of MCFA. However, CH_4 production expressed relative to digested OM decreased linearly ($p < .01$; $R^2 = 0.75$) by MCFA (Figure 2), but the levels of MCFA had no significant influence on gas production. Total VFA concentration tended to decrease linearly ($p = .07$; $R^2 = 0.87$) with MCFA supplementation. Dietary MCFA decreased the proportion of acetate (C_2; $p = .02$) with R^2 at 0.66 and tended to decrease the proportion of butyrate (C_4; $p = .08$) with R^2 at 0.82, but did not significantly alter the proportions of isobutyrate and isovalerate ($IsoC_4$ and

TABLE 3 Descriptive statistics of the variables in the database used to evaluate the effect of medium-chain fatty acids (MCFA) on ruminal methane production in ruminants

	In vitro					In vivo				
Item	*n*	Mean	*SD*	Max	Min	*n*	Mean	*SD*	Max	Min
DMI (kg/d)						65	10.2	7.9	26.5	0.7
Digestibility (%)										
DMD	23	56.4	12.4	81.5	38.1	32	66.3	6.3	75.5	50.0
OMD	44	55.8	10.5	77.9	33.4	44	69.7	4.4	76.4	59.3
CPD	28	60.1	17.3	89.4	34.1	32	66.2	8.7	80.4	50.0
NDFD	53	25.6	13.5	57.3	5.3	46	55.6	7.9	70.0	35.2
Rumen fermentation										
pH	86	6.8	0.2	7.2	6.3	32	6.6	0.3	7.0	6.1
Gas (mL/g DMs)	63	218	120	489	9.0					
CH_4 (mL/d and L/d)	102	6.9	5.8	20.2	0.0	60	303	272	835	10.5
CH_4/DMs (L/kg)	110	14.4	11.3	45.4	0.2	53	31.1	11.0	54.5	9.6
CH_4/OMD (L/kg)	38	17.8	7.4	29.6	4.4	36	38.9	14.5	57.8	6.5
Total VFA (mmol/L)	106	72.7	31.3	170.7	21.0	36	101.2	16.6	134.9	59.5
C_2 (mol/100 mol VFA)	108	56.9	6.0	70.2	40.2	36	65.8	2.5	70.1	60.1
C_3 (mol/100 mol VFA)	109	22.6	6.8	39.5	11.4	40	21.2	3.0	27.1	13.8
C_4 (mol/100 mol VFA)	109	13.9	4.3	24.2	5.1	40	10.4	2.4	15.3	6.3
IsoC_4 (mol/100 mol VFA)	66	1.1	0.9	2.6	0.1	27	0.7	0.4	1.2	0.1
C_5 (mol/100 mol VFA)	88	4.0	2.7	9.6	0.6	27	1.7	0.5	2.7	1.0
IsoC_5 (mol/100 mol VFA)	69	2.8	1.5	5.8	0.5	27	1.3	0.5	2.1	0.4
C_2:C_3	105	2.7	1.1	5.3	0.1	38	3.2	0.5	4.2	2.3
H_2 (mmol/L)	56	0.9	1.1	4.0	0.0					
NH_3 (mmol/L)	70	12.6	6.7	25.6	2.1	23	8.3	3.5	18.0	2.5
Microorganism										
Protozoa (10^4/ml)	49	0.51	0.54	2.24	0.0	34	51.7	36.6	138.2	1.0
Bacteria (10^9/ml)	40	3.3	1.5	5.1	0.3	20	17.7	14.5	44.0	3.0
Archaea (10^7/ml)	50	11.5	8.4	33.9	0.0	16	71.0	61.6	234.0	5.1

Abbreviation: *n*, number of observations; *SD*, standard deviation; Max, maximum; Min, minimum; DMD, digested dry matter; OMD, digested organic matter; CPD, digested crude protein; NDFD, digested neutral detergent fibre; CH_4:methane, mL/d for the in vitro and L/d for the in vivo; DMs, dry matter substrate for in vitro or dry matter intake for in vivo; VFA, volatile fatty acids; C_2, acetate; C_3, propionate; C_4, butyrate; Iso-C_4, isobutyrate; C_5, valerate; Iso-C_5:isovalerate; H_2, hydrogen.

IsoC_5). The proportion of propionate (C_3) showed a positive slope (p = .05) in a quadratic manner with an R^2 of 0.79. The proportion of valerate (C_5) tended to show a positive slope (p < .07; R^2 = 0.88). The increased C_3 levels resulted in a decrease in the C_2:C_3 ratio (p = .03; R^2 = 0.70). The concentration of NH_3 tended to decrease with increasing dietary concentrations of MCFA (p = .08; R^2 = 0.85). Populations of protozoa (p < .01) responded negatively in a linear manner while *Archaea* (p = .04) in a quadratically manner, with R^2 values of 0.65 and 0.71, respectively. The bacterial population was not affected by MCFA in vitro.

CO, LA and KO decreased the degradability of DM (23%–33%), while CO, LA and PKO decreased the degradability of NDF (29%–51%) compared with the control (p < .01; Table 5). LMA and LA effectively lowered CH_4 emissions expressed as millilitres (p < .01) by 42%–51%. LMA, LA, PKO and CO treatments also decreased (p < .01) CH_4 production per unit of digested OM by 32%–42% compared with the control. Although none of the dietary MCFA was able to change ruminal total VFA concentration, the C_3 proportion tended to increase with the CO and LA treatments (p = .07), while the C_5 proportion increased for LMA (p = .03). The LA treatments reduced the C_2:C_3 ratio, as compared with the control treatment (p = .03). MCFA treatment reduced the populations of protozoa (LA, CanO, CO and LMA treatments) and *Archaea* (LA and LMA treatments) by 68%–85% and 90%–100%, respectively (p < .01). However, there were no MCFA treatments capable of decreasing the bacterial population.

3.2 | In vivo experiments

The CH_4 emissions decreased with increasing concentrations of dietary MCFA in the in vivo experiments, when CH_4 production was expressed as L/d (linearly, $p < .01$ and $R^2 = 0.94$) and L/kg DMI (quadratically, $p = .05$, and $R^2 = 0.87$; Table 6). The CH_4 emissions also tended to decrease quadratically when CH_4 production was expressed as L/kg OMD ($p = .07$; $R^2 = 0.84$; Figure 2). The dietary MCFA had no effect on DM or OM digestibility, but linearly decreased the digestibility of both CP ($p = .09$; $R^2 = 0.96$) and NDF ($p = .02$; $R^2 = 0.77$). Dietary MCFA tended to decrease the pH of the ruminal fluid linearly ($p = .08$; $R^2 = 0.83$) and the total VFA concentration quadratically ($p = .06$; $R^2 = 0.81$). However, increasing levels of dietary MCFA tended to linearly increase the C_3 proportion

TABLE 4 Regression equations of ruminal fermentation parameters on the concentrations of medium-chain fatty acids (MCFA) addition in the in vitro experiments

			Parameter estimates						
Response parameters	***n***	**Model**	**Intercept**	***SE* Intercept**	**Slope**	***SE* Slope**	***P* value**	**RMSE**	R^2
In vitro digestibility (%)									
DMD	23	L	61.5	2.77	−0.2	0.08	.02	9.97	0.32
OMD	44	L	57.6	3.28	−0.07	0.05	ns	5.32	0.74
CPD	28	L	57.7	8.39	−0.09	0.06	ns	6.19	0.87
NDFD	53	L	29.8	3.82	−0.23	0.06	<.01	7.15	0.71
Rumen fermentation									
pH	86	L	6.82	0.05	−0.00006	0.0004	ns	0.09	0.78
Gas (mL/g DMs)	63	L	209	35.6	−0.5	0.4	ns	66.7	0.68
CH_4 (mL)	102	L	9.51	1.6	−0.044	0.02	.03	3.55	0.62
CH_4/DMs (L/kg)	110	L	18	2.3	−0.13	0.03	<.01	6	0.72
CH_4/OMD (L/kg)	38	L	23.7	2.1	−0.2	0.03	<.01	3.66	0.75
Total VFA (mmol/L)	106	L	81.4	7.1	−0.1	0.1	.07	11.17	0.87
C_2 (mol/100 mol VFA)	108	Q	57.9	1.3	−0.1	0.04	.01	3.5	0.66
					0.0014	0.0006	.02		
C_3 (mol/100 mol VFA)	109	Q	21.6	1.5	0.1	0.04	<.01	3.13	0.79
					−0.001	0.001	.05		
C_4 (mol/100 mol VFA)	109	L	14.2	1	−0.015	0.008	.08	1.83	0.82
IsoC_4 (mol/100 mol VFA)	66	L	0.83	0.26	0.00002	0.0019	ns	0.32	0.86
C_5 (mol/100 mol VFA)	88	Q	3.55	0.68	0.023	0.012	.06	0.93	0.88
					−0.00029	0.00016	.07		
IsoC_5 (mol/100 mol VFA)	69	L	2.2	0.46	−0.0024	0.003	ns	0.48	0.89
C_2:C_3	105	Q	3.01	0.24	−0.021	0.007	<.01	0.6	0.7
					0.0002	0.0001	.03		
H_2 (mmol/L)	56	L	0.56	0.28	0.01	0.003	<.01	0.49	0.78
NH_3 (mmol/L)	70	Q	11.75	1.8	−0.11	0.05	.03	2.59	0.85
					0.0014	0.0008	.08		
Microorganism									
Protozoa (10^3/ml)	49	L	10.3	1.03	−0.19	0.03	<.01	3.16	0.65
Bacteria (10^9/ml)	40	L	3.27	0.49	−0.0009	0.006	ns	0.6	0.84
Archaea (10^7/ml)	50	Q	14.52	5.41	−0.77	0.26	.01	4.48	0.71
					0.01	0.01	.04		

Note: The model is tended to be significantly compatible at $p \leq .10$. The model is considered compatible at $p \leq .05$.

Abbreviations: C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; CH_4, methane; CPD, digested crude protein; DMD, digested dry matter; DMs, dry matter substrate; H_2, hydrogen; Iso-C_4, isobutyrate; Iso-C_5, isovalerate; Model, linear (L) or quadratic (Q); *n*, number of observations; NDFD, digested neutral detergent fibre; OMD, digested organic matter; R^2, *R*-square;RMSE, root mean square of errors; *SE*, standard error; VFA, volatile fatty acids.

(p = .06; R^2 = 0.70) and quadratically increase the C_5 proportion (p = .02; R^2 = 0.98), while having no effect on C_2 proportion. As a result, the C_2:C_3 ratio also decreased slightly (p = .05) in a linear manner with an R^2 of 0.52. The concentrations of iso-C_4 and iso-C_5 decreased linearly (p = .03 and p < .01, respectively) with R^2 values of 0.91 and 0.87, respectively. The concentration of NH_3 in the ruminal fluid decreased (p < .01) quadratically with increasing levels of MCFA (R^2 = 0.93). The protozoa population responded negatively (p < .01; and R^2 = 0.73) in a linear manner, but no significant effect on *Archaea* population was noted with the increasing levels of MCFA.

The effects of MCFA in the in vivo experiments were categorized by source type (Table 7). The addition of CO and LA decreased the daily DM intake of the animals by 14%–15% (p = .01), but there was no significant effect on apparent nutrient digestibility by MCFA treatments, except for a slight decrease in digested DM (4%). Only the MA treatment decreased pH, compared to the control. CO treatment substantially lowered the CH_4 emissions by 28% when CH_4 emission was expressed in L/d (p = .04). Methane production expressed as L/kg DM intake was significantly lower by 21% to 38% due to feeding of CO and MA, compared with the control (p < .01). CH_4 production expressed as L/kg digested OM was decreased by 23% (p < .01) due to CO. The dietary MA decreased VFA concentration by 25%, and CO treatment decreased it by 12% compared with the control (p < .01).

The MA treatment caused a lesser concentration of C_4 and iso-C_4 than in the control (p = .02 and p = .01, respectively). Compared to the control, only CO increased C_3 proportion (p = .01) and C_5 proportion (p = .01). All MCFA treatments decreased the C_2:C_3 ratio (p = .008). MA and CO sources decreased ammonia concentration in the rumen by 25%–38% (p < .01), while LMA treatment increased the ammonia concentration by 87%, compared to the control. Of the MCFA treatments, only the LA, CO and MA sources reduced the protozoal population significantly (p < .01), but did not significantly affect total bacteria or *Archaea* populations.

4 | DISCUSSION

Our study employs a quantitative approach that may explain contrasting findings of the MCFA effects obtained from individual

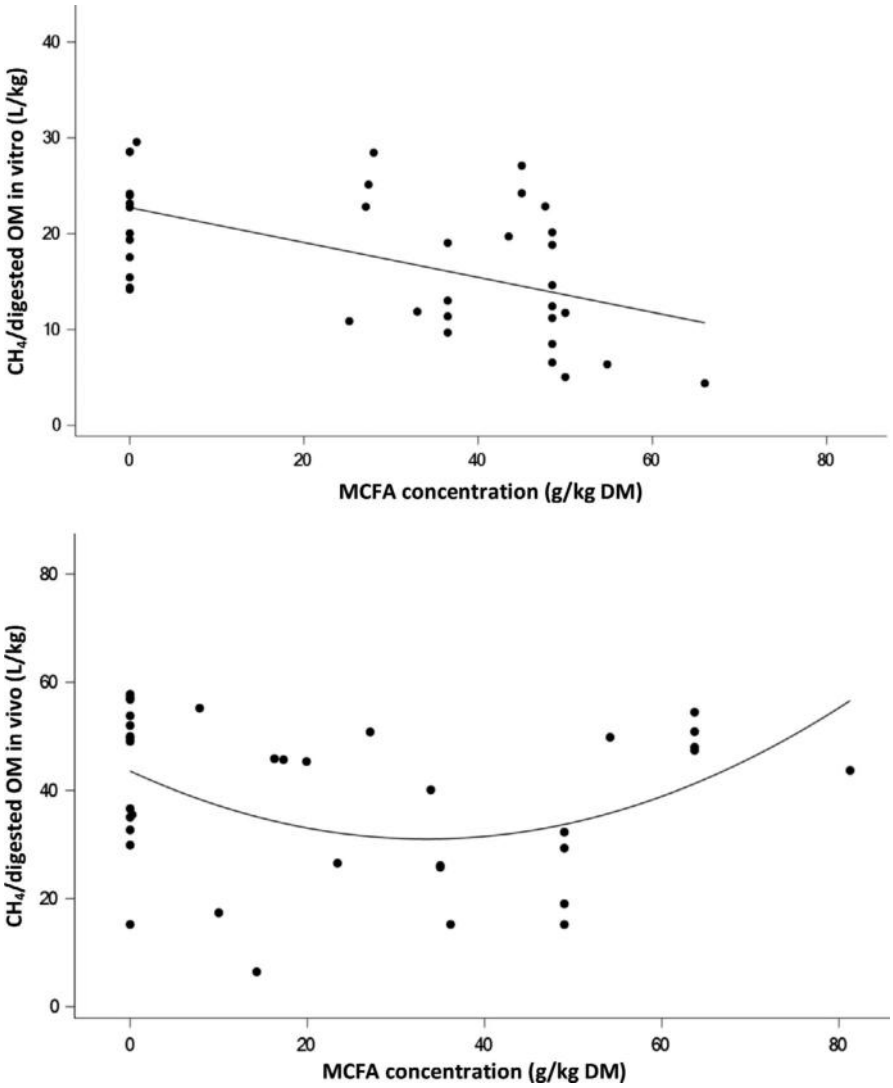

FIGURE 2 Relationship between the concentrations of medium-chain fatty acids (MCFA; g/kg DM) and CH_4 production per unit of digested OM (L/kg) in the in vitro experiments (upper panel) with a linear response and in vivo experiments (lower panel) with a quadratic response

TABLE 5 Responses of ruminal fermentation parameters affected by different medium-chain fatty acids (MCFA) sources in the in vitro experiments

Response parameters	*n*	Control	CO	CanO	KO	LA	MA	LMA	PKO	*p* value
In vitro digestibility (%)										
DMD	23	70.4^{a}	50.3^{b}	–	54.2^{b}	47.5^{b}	–	–	–	<.01
OMD	44	57.5	55.9	57.5	–	51.5	57.4	56.2	55.4	ns
CPD	28	58.0	51.9	–	–	53.7	59.0	53.2	–	ns
NDFD	50	30.5^{a}	18.1^{b}	21.8^{ab}	25.5^{ab}	15.0^{b}	27.9^{ab}	21.3^{ab}	15.3^{b}	<.01
Rumen fermentation										
pH	84	6.82	6.79	6.83	6.66	6.82	6.83	6.83	6.82	ns
Gas (mL/g DMs)	63	220	156	–	197	176	258	236	163	ns
CH_4 (mL)	102	9.75^{a}	10.2^{a}	–	9.21^{abc}	5.70^{b}	12.8^{ab}	4.77^{c}	–	<.01
CH_4/DMs (L/kg)	110	18.5^{a}	12.3^{b}	15.8^{ab}	11.9^{ab}	10.9^{b}	18.6^{ab}	13.4^{ab}	13.5^{ab}	<.01
CH_4/OMD (L/kg)	38	23.0^{a}	15.8^{b}	21.6^{ab}	–	13.7^{b}	21.9^{ab}	13.3^{b}	15.6^{b}	<.01
Total VFA (mmol/L)	106	81.7	76.7	80.2	80.6	75.2	85.9	79.1	82.2	ns
C_2 (mol/100 mol VFA)	105	57.9	56.2	58.0	55.9	56.3	59.5	54.4	56.2	ns
C_3 (mol/100 mol VFA)	109	21.5^{b}	24.4^{a}	21.3^{ab}	24.6^{ab}	24.3^{a}	22.1^{ab}	22.5^{ab}	23.5^{ab}	.07
C_4 (mol/100 mol VFA)	108	14.1	14.0	14.9	14.2	13.2	13.1	15.0	14.5	ns
$IsoC_4$ (mol/100 mol VFA)	66	0.86	0.64	–	–	0.84	0.85	0.80	–	ns
C_5 (mol/100 mol VFA)	79	3.57^{b}	2.89^{b}	4.42^{ab}	–	3.21^{b}	3.66^{b}	5.84^{a}	3.85^{b}	.03
Iso-C_5 (%)	69	2.28	1.62	–	–	2.18	2.06	2.06	1.65	ns
C_2:C_3	105	3.04^{a}	2.74^{ab}	3.45^{a}	2.61^{ab}	2.33^{b}	2.89^{ab}	2.71^{ab}	2.99^{ab}	.03
H_2 (mmol/L)	50	0.53^{c}	0.77^{abc}	0.53^{bc}	–	1.37^{ab}	0.49^{c}	1.84^{a}	1.24^{abc}	.02
NH_3 (mmol/L)	70	11.7	9.32	10.6	–	10.0	13.2	9.23	9.52	ns
Microorganism										
Protozoa (10^3/ml)	42	10.3^{a}	2.59^{b}	2.21^{b}	–	1.52^{b}	9.10^{a}	3.26^{b}	–	<.01
Bacteria (10^9/ml)	40	3.30	2.98	3.45		3.39	3.55	3.30	4.00	ns
Archaea (10^7/ml)	50	15.1^{a}	6.80^{ab}	9.75^{ab}		1.48^{b}	9.10^{a}	0.00^{b}	9.74^{ab}	<.01

Note: Different superscript alphabets of means in a row are significant differences at $p \leq .05$.

Abbreviations: C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; CanO, canola oil enriched with lauric acid; CH_4, methane; CO, coconut oil; CPD, digested crude protein; DMD, digested dry matter; DMs, dry matter substrate; H_2, hydrogen; Iso-C_4, isobutyrate; Iso-C_5, isovalerate; KO, krabok oil; LA, lauric acid; LMA, mixed lauric and myristic acids; MA, myristic acid; *n*, number of observations; NDFD, digested neutral detergent fibre; OMD, digested organic matter; PKO, palm kernel oil; VFA, volatile fatty acids.

experiments. The consistency of MCFA effects needs to be verified across various MCFA sources and experimental techniques. Although each MCFA source has a different effectiveness in mitigating CH_4 (Wang, Kreuzer, Braunb, & Schwarma, 2017), we have attempted to explain the general effect of MCFA on CH_4 mitigation. The effectiveness of different sources of MCFA in reducing CH_4 production is also discussed in the following sections.

4.1 | Effect of MCFA on rumen methanogenesis and fermentation

In general, saturated MCFA sources are capable of inhibiting the activity of microbes found in the rumen, including the protozoa, bacteria and methanogens which are responsible for methanogenesis (Božic et al., 2009; Kang et al., 2017), though the strength of this effect depends on the MCFA source. Supplementation with different MCFA sources (LA, MA or combinations therefore) has been suggested as an alternative strategy to reduce CH_4 production in the rumen (Hristov et al., 2011; Kim et al., 2014; Machmüller, 2006). It is, thus, necessary to employ meta-analysis to understand the underlying general effects of MCFA on ruminal methanogenesis.

Methane emission decreased with dietary MCFA supplementation, even when expressed per unit of OM digested. These results may be attributed to the direct inhibition of methanogenesis by MCFA, as well as to the reduction and activities of ruminal protozoa and/or *Archaea* populations. In accordance with CH_4 mitigation, digested NDF also decreased with dietary MCFA in both in vitro and in vivo experiments, because MCFA has an adverse effect on fibre digestion (Dohme et al., 1999; Dohme, Machmüller, Wasserfallen,

TABLE 6 Regression equations of ruminal fermentation parameters on the concentrations of medium-chain fatty acids (MCFA) addition in the in vivo experiments

			Parameter estimates						
Response Parameters	***n***	**Model**	**Intercept**	***SE* Intercept**	**Slope**	***SE* Slope**	***p* value**	**RMSE**	R^2
DMI (kg/d)	65	Q	10.2	1.9	−0.072	0.024	<.01	1.05	0.98
					0.0008	0.0004	.05		
In vivo digestibility (%)									
DMD	32	L	64.4	2.1	−0.005	0.02	ns	2.01	0.89
OMD	44	L	69.5	1.2	−0.01	0.02	ns	1.75	0.83
CPD	32	L	65.8	3.3	0.03	0.02	.09	1.61	0.96
NDFD	46	L	56.8	2.1	−0.08	0.03	.02	3.73	0.77
Rumen fermentation									
pH	32	L	6.61	0.1	−0.002	0.001	.08	0.12	0.83
CH_4 (L/d)	60	L	303	66.6	−1.58	0.5	<.01	67.6	0.94
CH_4/DMI (L/kg)	53	Q	33.8	2.79	−0.32	0.1	<.01	3.89	0.87
					0.0029	0.0014	.05		
CH_4/OMD (L/kg)	36	Q	42.2	4.57	−0.44	0.16	.01	5.67	0.84
					0.0048	0.0026	.07		
Total VFA (mmol/L)	36	Q	105.5	5.3	−0.58	0.29	.06	7.06	0.81
					0.011	0.005	.06		
C_2 (mol/100 mol VFA)	36	L	65.6	0.7	−0.011	0.013	ns	1.28	0.73
C_3 (mol/100 mol VFA)	40	L	20.5	0.8	0.03	0.02	.06	1.65	0.7
C_4 (mol/100 mol VFA)	40	L	10.8	0.7	−0.01	0.01	ns	1.02	0.81
IsoC_4 (mol/100 mol VFA)	27	L	0.78	0.13	−0.003	0.001	.03	0.1	0.91
C_5 (mol/100 mol VFA)	27	Q	1.69	0.2	0.01	0.004	.02	0.08	0.98
					−0.0002	0.0001	.02		
IsoC_5 (mol/100 mol VFA)	27	L	1.46	0.19	−0.006	0.002	<.01	0.18	0.87
C_2:C_3	38	L	3.27	0.12	−0.006	0.003	.05	0.31	0.52
NH_3 (mmol/L)	23	L	10.5	1.3	−0.08	0.02	<.01	0.91	0.93
Microorganism									
Protozoa (10^5/ml)	34	L	7.14	0.99	−0.09	0.02	<.01	1.87	0.73
Bacteria (10^9/ml)	20	L	18	7.2	−0.05	0.04	ns	2.77	0.96
Archaea (10^7/ml)	16	L	88.5	32.9	−0.52	0.5	ns	31.2	0.73

Note: The model is tended to be significantly compatible at $p \leq .10$. The model is considered compatible at $p \leq .05$.

n, number of observations; Model, linear (L) or quadratic (Q); *SE*, standard error; RMSE, root mean square of errors; R^2, R-square; DMI, dry matter intake; DMD, digested dry matter; OMD, digested organic matter; CPD, digested crude protein; NDFD, digested neutral detergent fibre; CH_4, methane; VFA, volatile fatty acids; C_2, acetate; C_3, propionate; C_4, butyrate; Iso-C_4, isobutyrate; C_5, valerate; Iso-C_5, isovalerate.

& Kreuzer, 2000; Patra & Yu, 2013; Ushida, Umeda, Kishigami, & Kojima, 1992) and ruminal microbial populations that are responsible for degradation of fibrous substrates (Klevenhusen et al., 2009). Fatty acids can be adsorbed on the microbes or feed particles in the rumen (Patra, 2013; Patra & Yu, 2013). Since small MCFA molecules dissolve readily in the lipid layer of the cell membrane, they can efficiently cause physical disruption of cell membranes and interfere in energy metabolism and nutrient transport, leading to the death of certain microbial cells, such as cellulolytic bacteria and ciliate protozoa (Kang et al., 2017; Machmüller, 2006; Patra, 2013). Fibre degrading micro-organisms are among the most sensitive to dietary fats (Patra, 2013). The number of ruminal microbes was thus decreased by MCFA, as indicated by the diminished number of protozoa in the in vitro and in vivo studies. The strength of this antimicrobial effect is proportional to the MCFA concentration, which leads to a decrease in the growth of protozoa and methanogens (Debruyne et al., 2018; Machmüller, Soliva, & Kreuzer, 2003a). Although total *Archaea* population in the in vivo result was not

TABLE 7 Responses of ruminal fermentation parameters affected by different medium-chain fatty acids (MCFA) sources in the in vivo experiments

Response parameters	*n*	Control	CO	LA	MA	LMA	*p* value
DMI (kg/d)	65	10.3^{a}	8.90^{b}	8.75^{b}	10.2^{ab}	10.0^{ab}	.01
In vivo digestibility (%)							
DMD	30	67.8^{a}	64.8^{b}	69.3^{a}	–	–	.03
OMD	43	70.1	68.6	71.5	68.0	–	ns
CPD	32	65.8	66.7	66.6	–	–	ns
NDFD	46	57.5	53.0	57.9	53.4	60.4	ns
Rumen fermentation							
pH	32	6.58^{a}	6.64^{a}	6.56^{a}	6.15^{b}	–	<.01
CH_4 (L/d)	60	320^{a}	231^{b}	285^{ab}	296^{ab}	284^{ab}	.04
CH_4/DMI (L/kg)	53	34.6^{a}	27.4^{b}	33.7^{ab}	21.3^{bc}	27.0^{abc}	<.01
CH_4/OMD (L/kg)	36	42.9^{a}	33.0^{b}	41.5^{ab}	30.2^{ab}		<.01
Total VFA (mmol/L)	34	112^{a}	99^{b}	110^{a}	84^{c}	111^{ab}	<.01
C_2 (mol/100 mol VFA)	37	65.8	65.2	64.4	65.6	62.3	ns
C_3 (mol/100 mol VFA)	40	20.1^{b}	21.0^{ab}	22.3^{ab}	24.6^{a}	23.2^{ab}	.02
C_4 (mol/100 mol VFA)	40	10.8^{a}	11.1^{a}	10.6^{a}	7.5^{b}	10.1^{ab}	.02
IsoC_4 (mol/100 mol VFA)	27	0.77^{a}	0.79^{a}	0.71^{a}	0.40^{b}	0.70^{ab}	.01
C_5 (mol/100 mol VFA)	24	1.68^{bc}	1.88^{a}	1.68^{b}	1.49^{c}	1.88^{ab}	.01
Iso-C_5 (mol/100 mol VFA)	27	1.47	1.23	1.34	1.06	1.68	ns
C_2:C_3	37	3.38^{a}	3.07^{b}	2.93^{bc}	2.51^{c}	2.74^{bc}	<.01
NH_3 (mM)	23	9.61^{b}	7.25^{c}	7.33^{bc}	5.98^{c}	18.0^{a}	<.01
Microorganism							
Protozoa (10^5/ml)	34	8.46^{a}	4.24^{b}	1.25^{b}	4.78^{b}	6.33^{ab}	<.01
Bacteria (10^9/ml)	20	18.1	16.5	–	16.7	–	ns
Archaea (10^7/ml)	16	97.4	59.3	–	84.0	–	ns

Note: Different superscript alphabets of means in a row are significant differences at $p \leq .05$.

n, number of observations; CO, coconut oil; LA, lauric acid; MA, myristic acid; LMA, mixed lauric and myristic acids; DMI, dry matter intake; DMD, digested dry matter; OMD, digested organic matter; CPD, digested crude protein; NDFD, digested neutral detergent fibre; CH_4, methane; VFA, volatile fatty acids; C_2, acetate; C_3, propionate; C_4, butyrate; Iso-C_4, isobutyrate; C_5, valerate; Iso-C_5, isovalerate.

affected significantly, the *Archaea* activity might be inhibited due to the MCFA supplementation. Thus, remaining H_2 may be optimized by non-inhibited microbial populations to form C_3 through interspecies H_2 transfer (Machmüller, 2006; Wang et al., 2017). The present analysis indicates that the total bacterial population was not affected by dietary MCFA. The diminished protozoa and cellulolytic bacteria by the dietary MCFA and adsorption of MCFA on the feed particles may decrease ruminal fibre digestibility. Fibre degradation decreased to a lesser extent in the in vivo studies compared with the in vitro studies. These differences were indicated by their NDFD's slope values (−0.08% versus −0.23%, respectively) and their intercept values (56.8% versus 29.8%, respectively). Because of observed values, we can conclude that the fibre degradation due to MCFA supplementation decreased deeper in vitro than in vivo. However, effectivity of fibre degradation activity due to MCFA finally decreased in both in vitro and in vivo conditions. The addition of the MCFA could reduce OM and fibre digestibility, which was attributed to changes in the microbial communities, a reduction in fermentative activity or the limitation of the access of microbes and enzymes to feed particles due to physical coating (Nagaraja, Newbold, van Nevel, & Demeyer, 1997; Vargas et al., 2017). The number of *Archaea* was diminished in the in vitro studies by MCFA, but not in the in vivo studies. Consequently, the C_2 production was not affected by the MCFA in vivo (Table 6) and the pool of H_2 sink might be conditioned (Moss, Jouany, & Newbold, 2000; Wang et al., 2018). *Archaea* require H_2 as an energy source; thus, parallel with the C_2 production, the *Archaea* population in the in vivo studies was not completely affected. However, it seems that the H_2 gas was not used effectively by *Archaea* for CH_4 production. CH_4 production was thus suppressed, and an increase in H_2 concentration was observed. Because H_2 accumulation is not stoichiometric with the inhibition of CH_4 formation, the increasing amount of H_2 produced might have effect on VFA concentrations formation (Soliva et al., 2004). In the present study, VFA concentrations were significantly decreased only in the in vivo studies, but the proportion of C_3, both in vitro and in vivo experiments, was increased due to dietary MCFA. Higher concentration of C_3 resulted in a decrease in C_2:C_3 ratio under in vitro and in vivo conditions, which is usually noted when CH_4 inhibition

effects are mediated by many CH_4 inhibiting agents, including MCFA (Patra, 2016).

4.2 | The effectiveness of different MCFA sources on CH_4 mitigation

Most MCFA sources proved their effectiveness against methanogenesis, although the magnitude of inhibition varied depending upon the type and concentration of fat, dietary composition and animal species (Grainger & Beauchemin, 2011; Machmüller, 2006; Patra, 2014). Previous findings have shown that the addition of synthetic or monoester MCFA, such as LA or MA, was more effective in modulating ruminal fermentation, acting as strong defaunation agents in the rumen (Dohme, Machmüller, Wasserfallen, & Kreuzer, 2001; Nguyen & Hegarty, 2017). The present study has shown that CO and MA effectively suppress CH_4 production in in vivo experiments by as much as 21% and 38%, respectively, but that LA diets were less effective in decreasing ruminal CH_4 emission. However, in vitro CH_4 emission was decreased by up to 42% by dietary LA, and dietary LMA, CO and PKO were also effective in suppressing CH_4 (about 27%–51%). It seems that there may have been a synergistic effect of LA and MA on the CH_4-suppressing effect, as the mixture of LA and MA strongly decreased CH_4 formation (Soliva, Hindrichsen, Meile, Kreuzer, & Machmüller, 2003). Among the various MCFA sources, only the addition of CO, LA, LMA and PKO decreased CH_4 production per unit of digested OM in the in vitro experiments (31% to 42%), as compared to the control; in the in vivo experiments, the addition of MA and CO also reduced CH_4 production (18% to 20%). LA and CO also potentially reduced the number of rumen protozoa that adversely affect NDF digestibility, leading to greater inhibition of methanogenesis (Faciola & Broderick, 2014).

The anti-methanogenic effect of MCFA is attributed to the inhibition of ruminal microbes, such as protozoa and methanogens (Machmüller, Soliva, & Kreuzer, 2003b; Soliva et al., 2004). However, our results show that the *Archaea* population in the in vivo studies was not significantly decreased by any MCFA treatment. Although CH_4 mitigation by the LA seemed more effective, the use of CO in the in vivo experiments was more effective in reducing CH_4 emissions per unit of digested OM. Nevertheless, combinations of LA and MA appear to be more effective in reducing CH_4 formation in the rumen (Dong, Bae, McAllister, Mathison, & Cheng, 1997; Soliva et al., 2004). Dohme et al. (2000) stated that sources rich in LA and MA, such as CO and PKO, in which the LA:MA ratio was approximately 2.5:1, significantly reduced methanogen and ciliate protozoa populations. The combined results from both in vitro and in vivo experiments suggest that CO consistently inhibited methanogenesis. Microbial sensitivity to particular MCFA is determined by the cell wall structures of microbes. Ciliate protozoa and gram-positive microbes are more strongly affected than gram-negative bacteria (Hovorková, Laloučková, & Skřivanová, 2018; Machmüller et al., 2003b). After the inhibition of certain sensitive rumen microbes, the remaining uninhibited microbial populations determine the extent of the hydrogen-producing and hydrogen-utilizing processes in the rumen. The present study showed no effect on the in vitro VFA concentration, except that in in vivo studies, MA and CO caused (25% and 12%, respectively) lower VFA concentration than in the control. In the in vivo studies, LA, LMA and CO showed no significant differences in VFA concentrations compared with the control, although these sources have the potential to decrease ruminal *Archaea* populations. This indicated the different responses of *Archaea* populations among treatment groups in both the in vitro and in vivo experiments. This bias might be attributed to different counting techniques and limited number of observations. Moreover, the fermentation products of CO treatment seem to be lower due to the other synergetic mechanisms of MCFA sources, where decreased ruminal microbes are responsible for decreased CH_4 formation and digestibility, leading to lowered VFA concentrations. Thus, appropriate amounts of mixed LA and MA may alter methanogenic populations.

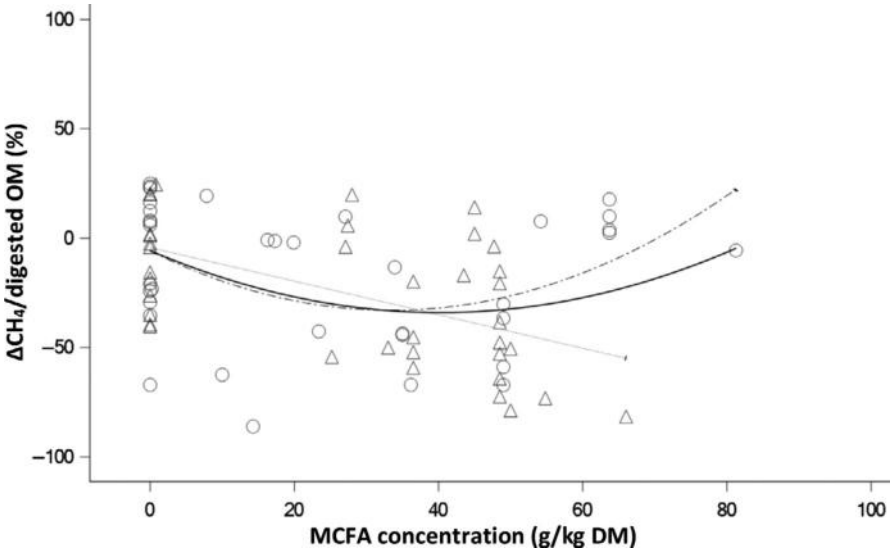

FIGURE 3 Relative changes in methane production with dietary medium-chain fatty acids (MCFA) concentrations in vitro and in vivo. Methane production (L/kg organic matter digested) converted to percentage change was predicted based on MCFA concentration (g/kg DM) in the in vitro (Δ, dot regression line) and in vivo (O, dash regression line) experiments. The solid regression line represents for both in vitro and in vivo experiments

4.3 | 3 Robustness of in vitro methods in predicting the in vivo relationship of dietary MCFA to CH_4 emission

Short-term and long-term in vitro fermentation experiments may be helpful in predicting the effects of MCFA on in vivo rumen fermentation parameters. In the in vivo system, the direct absorption and continuous flow of substrates and outflow of products occur. Thus, in vitro experiments have limitations in reflecting the effect of dietary MCFA on ruminal CH_4 emission. The relationship between dietary MCFA levels and CH_4 emission can be extrapolated using similar units for the in vitro and in vivo techniques. This is based on the proportional change of CH_4/digested OM, which was converted from mL/g and L/kg units to a percentage unit by taking the intercept as the reference value (Figure 3).

The regression model suggests that the relationship of the CH_4 changes/digested OM with the dietary MCFA concentration in the in vitro and in vivo approaches was low (R^2 = 0.17). Although the CH_4 changes/digested OM regression model was significant in a quadratic manner (p < .01), the intercept value was not significant (p = .47). The poor relationship between in vitro and in vivo studies in ruminal methanogenesis suggests that in vitro methods are limited in their ability to effectively simulate in vivo conditions (Flachowsky & Lebzien, 2009; Jayanegara et al., 2012; Krishnamoorthy, Rymer, & Robinson, 2005). The poor relationship (R^2 of 0.26) between in vitro and in vivo has also been reported by Moss and Givens (1997). However, the in vitro results might be extrapolated to the in vivo measurements by evaluating the intercept values of both experimental systems (Jayanegara et al., 2012). The intercepts of CH_4 emission/digested OM (in ml/g; i.e. substrate without dietary MCFA) were 23.7 and 42.2, respectively, in the in vitro and in vivo approaches (Figure 2). The in vitro intercept was still lesser than the in vivo value. When the relationship expressed in CH_4/DM substrate in the in vitro and CH_4/DM intake for the in vivo studies was evaluated, the intercepts (18.0 and 33.8, respectively) between them were still not similar. However, the decrease in CH_4 caused by dietary MCFA was apparent in both in vitro and in vivo studies. Although both in vitro and in vivo CH_4 values decreased with MCFA additions to the diets, CH_4 mitigation in the in vivo measurements was optimum at < 40 g/kg DM of MCFA, whereas the reduction of in vitro CH_4 emission/digested OM showed a linear decrease (Figure 3). It could be stated that in vitro results do not fully reflect the in vivo regarding the effect of MCFA on rumen methane production.

5 | CONCLUSION

Increasing the MCFA concentrations caused a decrease in CH_4 emission in both in vitro and in vivo studies, and this was associated with diminished populations of protozoa both in the in vitro and in vivo studies and *Archaea* in the in vitro study, but not of bacteria. Among the sources of MCFA, suppression of CH_4 production was noted in the following order: coconut oil > lauric acid > myristic acid > mixed lauric and myristic acids > palm kernel oil > canola oil enriched with lauric acids > krabok oil. In general, greater concentrations of dietary MCFA resulted in the reduction of ruminal fermentation products and digestibility. The in vitro results did not fully reflect the in vivo results regarding the effect of MCFA on ruminal methane production. The effective dose of dietary MCFA is usually 40 g/kg DM to decrease methane production, with the exception of MA and KO due to limited numbers of observations for these MCFA sources.

ACKNOWLEDGEMENTS

The in vitro part of this work was supported by the statutory funding of the Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poland [506.533.04]; and the in vivo part by the National Science Center (Kraków, Poland) [UMO-2016/23/B/NZ9/03427]. Support was also provided by the Ministry of Science and Higher Education's Regional Initiative Excellence programme 2019–2022 [005/RID/2018/19]. YRY was awarded an Ignacy Lukasiewicz PhD scholarship from the Polish National Agency for Academic Exchange (NAWA; 015/IL/1617). HH is a PhD scholarship holder under grant 2016/23/B/NZ9/03427, funded by National Science Center, Poland.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal have been adhered to. No ethical approval was required as this is a meta-analysis article that used the results of published research data.

ORCID

Yulianri Rizki Yanza https://orcid.org/0000-0001-8433-7644
Małgorzata Szumacher-Strabel https://orcid.org/0000-0003-0086-5744
Anuraga Jayanegara https://orcid.org/0000-0001-7529-9770
Amlan Kumar Patra https://orcid.org/0000-0003-1410-0902
Adam Cieślak https://orcid.org/0000-0001-7518-579X

REFERENCES

Ajisaka, N., Mohammed, N., Hara, K., Mikuni, K., Hara, K., Hashimoto, H., … Itabashi, H. (2002). Effects of medium-chain fatty acid-cyclodextrin complexes on ruminal methane production in vitro. *Journal of Animal Science*, *73*, 479–484. https://doi.org/10.1046/j.1344-3941.2002.00066.x

Bhatta, R., Tajima, K., Takusari, N., Higuchi, K., Enishi, O., & Kurihara, M. (2007). Comparison of in vivo and in vitro techniques for methane production from ruminant diets. *Asian-Australasian Journal of Animal Sciences*, *20*(7), 1049–1056. https://doi.org/10.5713/ajas.2007.1049

Blaxter, K. L., & Czerkawski, J. (1966). Modification of the methane production of the sheep by supplementation of ITS diet. *Journal of the Science of Food and Agriculture*, *17*, 417–421. https://doi.org/10.1002/jsfa.2740170907

Bošnjak, R., Prpić, Z., Ugarković, N. K., Konjačić, M., & Vnučec, I. (2018). Possibilities for mitigating the environmental footprint of dairy ruminants. *Mljekarstvo*, *68*, 155–168. https://doi.org/10.15567/mljekarstvo.2018.0301

Božic, A. K., Anderson, R. C., Carstens, G. E., Ricke, S. C., Callaway, T. R., Yokoyama, M. T., … Nisbet, D. J. (2009). Effects of the methane-inhibitors nitrate, nitroethane, lauric acid, Lauricidin ® and the Hawaiian marine algae Chaetoceros on ruminal fermentation in vitro. *Bioresource Technology*, *100*, 4017–4025. https://doi.org/10.1016/j.biortech.2008.12.061

Cieślak, A., Szumacher-Strabel, M., Szymankiewicz, E., Piękniewski, M., Oleszak, P., Siwiński, Ł., & Potkański, A. (2006). Coconut oil reduces protozoa count and methane release during fermentation in a Rusitec system. *Journal of Animal and Feed Sciences*, *15*, 19–22.

Crompton, L. A., Mills, J. A. N., & Reynolds, C. K. (2010). Effect of feeding frequency and replacing calcium salts of palm oil with crushed rapeseed or coconut oil on methane emissions in lactating dairy cows. *Proceedings of the Nutrition Society*, *69*(OCE4), E329. https://doi.org/10.1017/S0029665110001382

Czerkawski, J. W., & Breckenridge, G. (1977). Design and development of a long-term rumen simulation technique (Rusitec). *British Journal of Nutrition*, *38*(3), 371–384. https://doi.org/10.1079/BJN19770102

Debruyne, S., Ruiz-González, A., Artiles-Ortega, E., Ampe, B., Van Den Broeck, W., De Keyser, E., … Fievez, V. (2018). Supplementing goat kids with coconut medium chain fatty acids in early life influences growth and rumen papillae development until 4 months after supplementation but effects on in vitro methane emissions and the rumen microbiota are transient. *Journal of Animal Science*, *96*, 1978–1995. https://doi.org/10.1093/jas/sky070

Ding, X., Long, R., Zhang, Q., Huang, X., Guo, X., & Mi, J. (2012). Reducing methane emissions and the methanogen population in the rumen of Tibetan sheep by dietary supplementation with coconut oil. *Tropical Animal Health and Production*, *44*, 1541–1545. https://doi.org/10.1007/s11250-012-0103-7

Dohme, F., Machmüller, A., Estermann, B. L., Pfister, P., Wasserfallen, A., & Kreuzer, M. (1999). The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. *Letters in Applied Microbiology*, *29*, 187–192. https://doi.org/10.1046/j.1365-2672.1999.00614.x

Dohme, F., Machmüller, A., Wasserfallen, A., & Kreuzer, M. (2000). Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. *Canadian Journal of Animal Science*, *80*, 473–482. https://doi.org/10.4141/a99-113

Dohme, F., Machmüller, A., Wasserfallen, A., & Kreuzer, M. (2001). Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Letters in Applied Microbiology*, *32*, 47–51. https://doi.org/10.1046/j.1472-765X.2001.00863.x

Dong, Y., Bae, H. D., McAllister, T. A., Mathison, G. W., & Cheng, K. J. (1997). Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC). *Canadian Journal of Animal Science*, *77*, 269–278. https://doi.org/10.4141/A96-078

F. A. O., & World Health Organization. (2009). FAO/IAEA international symposium on sustainable improvement of animal production and health. Synopses (No. IAEA-CN–174). Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Faciola, A. P., & Broderick, G. A. (2014). Effects of feeding lauric acid or coconut oil on ruminal protozoa numbers, fermentation pattern, digestion, omasal nutrient flow, and milk production in dairy cows. *Journal of Dairy Science*, *97*, 5088–5100. https://doi.org/10.3168/jds.2013-7653

Flachowsky, G., & Lebzien, P. (2009). Comments on in vitro studies with methane inhibitors. *Animal Feed Science and Technology*, *151*, 337–339. https://doi.org/10.1016/j.anifeedsci.2009.02.003

Giger-Reverdin, S., & Sauvant, D. (2000). Methane production in sheep in relation to concentrate feed composition from bibliographic data. *Cahiers Options Méditerranéennes*, *52*, 43–46.

Grainger, C., & Beauchemin, K. A. (2011). Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology*, *166*, 308–320. https://doi.org/10.1016/j.anifeedsci.2011.04.021

Hollmann, M., Powers, W. J., Fogiel, A. C., Liesman, J. S., & Beede, D. K. (2013). Response profiles of enteric methane emissions and lactational performance during habituation to dietary coconut oil in dairy cows. *Journal of Dairy Science*, *96*, 1769–1781. https://doi.org/10.3168/jds.2012-6039

Hollmann, M., Powers, W. J., Fogiel, A. C., Liesman, J. S., Bello, N. M., & Beede, D. K. (2012). Enteric methane emissions and lactational performance of Holstein cows fed different concentrations of coconut oil. *Journal of Dairy Science*, *95*, 2602–2615. https://doi.org/10.3168/jds.2011-4896

Hovorková, P., Laloučková, K., & Skřivanová, E. (2018). Determination of in vitro antibacterial activity of plant oils containing medium-chain fatty acids against gram-positive pathogenic and gut commensal bacteria. *Czech Journal of Animal Science*, *63*, 119–125. https://doi.org/10.17221/70/2017-CJAS

Hristov, A. N., Lee, C., Cassidy, T., Long, M., Heyler, K., Corl, B., & Forster, R. (2011). Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science*, *94*, 382–395. https://doi.org/10.3168/jds.2010-3508

Hristov, A. N., Vander Pol, M., Agle, M., Zaman, S., Schneider, C., Ndegwa, P., … Karnati, S. K. R. (2009). Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *Journal of Dairy Science*, *92*, 5561–5582. https://doi.org/10.3168/jds.2009-2383

Jayanegara, A., Leiber, F., & Kreuzer, M. (2012). Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. *Journal of Animal Physiology and Animal Nutrition*, *96*, 365–375. https://doi.org/10.1111/j.1439-0396.2011.01172.x

Jayanegara, A., Wina, E., & Takahashi, J. (2014). Meta-analysis on methane mitigating properties of saponin-rich sources in the Rumen: Influence of addition levels and plant sources. *Asian-Australasian Journal of Animal Sciences*, *27*, 1426–1435. https://doi.org/10.5713/ajas.2014.14086

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Sciences*, *73*, 2483–2492. https://doi.org/10.2527/1995.7382483x

Jordan, E., Lovett, D. K., Hawkins, M., Callan, J. J., & O'Mara, F. P. (2006a). The effect of varying levels of coconut oil on intake, digestibility and methane output from continental cross beef heifers. *Journal of Animal Sciences*, *82*, 859–865. https://doi.org/10.1017/ASC2006107

Jordan, E., Lovett, D. K., Monahan, F. J., Callan, J., Flynn, B., & O'Mara, F. P. (2006b). Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *Journal of Animal Sciences*, *84*, 162–170. https://doi.org/10.2527/2006.841162x

Kang, S., Wanapat, M., Phesatcha, K., Norrapoke, T., Foiklang, S., Ampapon, T., & Phesatcha, B. (2017). Using krabok (Irvingia malayana) seed oil and Flemingia macrophylla leaf meal as a rumen enhancer in an in vitro gas production system. *Animal Production Science*, *57*, 327–333. https://doi.org/10.1071/AN15211

Kang, S., Wanapat, M., & Viennasay, B. (2016). Supplementation of banana flower powder pellet and plant oil sources on in vitro ruminal fermentation, digestibility, and methane production. *Tropical Animal Health and Production*, *48*, 1673–1678. https://doi.org/10.1007/s11250-016-1142-2

Kim, D. H., Mizinga, K. M., Kube, J. C., Friesen, K. G., McLeod, K. R., & Harmon, D. L. (2014). Influence of monensin and lauric acid distillate

or palm oil on in vitro fermentation kinetics and metabolites produced using forage and high concentrate substrates. *Animal Feed Science and Technology*, *189*, 19–29. https://doi.org/10.1016/j.anifeedsci.2013.12.010

Klevenhusen, F., Bernasconi, S. M., Hofstetter, T. B., Bolotin, J., Kunz, C., & Soliva, C. R. (2009). Efficiency of monolaurin in mitigating ruminal methanogenesis and modifying C-isotope fractionation when incubating diets composed of either C3 or C4 plants in a rumen simulation technique (Rusitec) system. *British Journal of Nutrition*, *102*, 1308–1317. https://doi.org/10.1017/S0007114509990262

Klop, G., Dijkstra, J., Dieho, K., Hendriks, W. H., & Bannink, A. (2017). Enteric methane production in lactating dairy cows with continuous feeding of essential oils or rotational feeding of essential oils and lauric acid. *Journal of Dairy Science*, *100*, 3563–3575. https://doi.org/10.3168/jds.2016-12033

Knapp, J. R., Laur, G. L., Vadas, P. A., Weiss, W. P., & Tricarico, J. M. (2014). Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science*, *97*, 3231–3261. https://doi.org/10.3168/jds.2013-7234

Kongmun, P., Wanapat, M., Nontaso, N., Nishida, T., & Angthong, W. (2009). Effect of phytochemical and coconut oil supplementation on rumen ecology and methane production in ruminants. FAO/IAEA international symposium: Sustainable Improvement of Animal Production and Health (IAEA-CN–174), 246–247.

Krishnamoorthy, U., Rymer, C., & Robinson, P. H. (2005). The in vitro gas production technique: Limitations and opportunities. *Animal Feed Science and Technology*, *123-124*, 1–7. https://doi.org/10.1016/j.anifeedsci.2005.04.015

Liu, H., Vaddella, V., & Zhou, D. (2011). Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. *Journal of Dairy Science*, *94*, 6069–6077. https://doi.org/10.3168/jds.2011-4508

Lovett, D., Lovell, S., Stack, L., Callan, J., Finlay, M., Conolly, J., & O'Mara, F. P. (2003). Effect of forage/concentrate ratio and dietary coconut oil level on methane output and performance of finishing beef heifers. *Livestock Production Science*, *84*, 135–146. https://doi.org/10.1016/j.livprodsci.2003.09.010

Machmüller, A. (2006). Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. *Agriculture, Ecosystems & Environment*, *112*, 107–114. https://doi.org/10.1016/j.agee.2005.08.010

Machmüller, A., Dohme, F., Soliva, C. R., Wanner, M., & Kreuzer, M. (2001). Diet composition affects the level of ruminal methane suppression by medium-chain fatty acids. *Australian Journal of Agricultural Research*, *52*(7), 713–722. https://doi.org/10.1071/AR00073

Machmüller, A., & Kreuzer, M. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science*, *79*, 65–72. https://doi.org/10.4141/A98-079

Machmüller, A., Ossowski, D. A., & Kreuzer, M. (2000). Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. *Animal Feed Science and Technology*, *85*, 41–60. https://doi.org/10.1016/S0377-8401(00)00126-7

Machmüller, A., Ossowski, D. A., Wanner, M., & Kreuzer, M. (1998). Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). *Animal Feed Science and Technology*, *71*, 117–130. https://doi.org/10.1016/S0377-8401(97)00126-0

Machmüller, A., Soliva, C. R., & Kreuzer, M. (2002). In vitro ruminal methane suppression by lauric acid as influenced by dietary calcium. *Canadian Journal of Animal Science*, *82*, 233–239. https://doi.org/10.4141/A01-078

Machmüller, A., Soliva, C. R., & Kreuzer, M. (2003a). Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. *British Journal of Nutrition*, *90*, 529–540. https://doi.org/10.1079/bjn2003932

Machmüller, A., Soliva, C. R., & Kreuzer, M. (2003b). Effect of coconut oil and defaunation treatment on methanogenesis in sheep. *Reproduction Nutrition Development*, *43*, 41–55. https://doi.org/10.1051/rnd:2003005

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. *Journal of Agricultural Science*, *93*, 217–222. https://doi.org/10.1017/S0021859600086305

Moss, A. R., & Givens, D. I. (1997). Methane production from a range of feedstuffs as determined in vitro using the cumulative gas production technique and compared with that measured in vivo. *Proceedings of the British Society of Animal Science*, 194.

Moss, A. R., Jouany, J. P., & Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. *Annales De Zootechnie.*, *49*, 231–253.

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., & Koch, D. (2013). Anthropogenic and natural radiative forcing, in Climate Change 2013: The Physical Science Basis. In T. F. Stocker, et al. (Ed.), *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 659–740), Cambridge and New York, NY: Cambridge University Press.

Nagaraja, T. G., Newbold, C. J., van Nevel, C. J., & Demeyer, D. I. (1997). Manipulation of ruminal fermentation. In P. N. Hobson, & C. S. Stewart (Eds.), *The Rumen Microbial Ecosystem* (pp. 523–632). Dordrecht: Springer.

Nguyen, S. H., & Hegarty, R. S. (2017). Effects of defaunation and dietary coconut oil distillate on fermentation, digesta kinetics and methane production of Brahman heifers. *Journal of Animal Physiology and Animal Nutrition*, *101*, 984–993. https://doi.org/10.1111/jpn.12534

O'Brien, M., Navarro-Villa, A., Purcell, P. J., Boland, T. M., & O'Kiely, P. (2014). Reducing in vitro rumen methanogenesis for two contrasting diets using a series of inclusion rates of different additives. *Animal Production Science*, *54*, 141–157. https://doi.org/10.1071/AN12204

Patra, A. K. (2009). A meta-analysis on effects of supplementing low-quality roughages with foliages from browses and tree fodders on intake and growth in sheep. *Livestock Science*, *121*, 239–249. https://doi.org/10.1016/j.livsci.2008.06.020

Patra, A. K. (2013). The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. *Livestock Science*, *155*, 244–254. https://doi.org/10.1016/j.livsci.2013.05.023

Patra, A. K. (2014). A meta-analysis of the effect of dietary fat on enteric methane production, digestibility and rumen fermentation in sheep, and a comparison of these responses between cattle and sheep. *Livestock Science*, *162*, 97–103. https://doi.org/10.1016/j.livsci.2014.01.007

Patra, A. K. (2016). Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. *Frontiers in Veterinary Science*, *3*, 1–17. https://doi.org/10.3389/fvets.2016.00039

Patra, A. K., Park, T., Kim, M., & Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *Journal of Animal Science and Biotechnology*, *8*, 1–18. https://doi.org/10.1186/s40104-017-0145-9

Patra, A. K., & Yu, Z. (2013). Effects of coconut and fish oils on ruminal methanogenesis, Fermentation, And abundance and diversity of microbial populations in vitro. *Journal of Dairy Science*, *96*, 1782–1792. https://doi.org/10.3168/jds.2012-6159

Pilajun, R., Wanapat, M., Wachirapakorn, C., & Navanukroaw, C. (2010). Effect of coconut oil and sunflower oil ratio on ruminal fermentation, rumen microorganisms, N-balance and digestibility in cattle. *Journal*

of Animal and Veterinary Advances, *9*(13), 1868–1874. https://doi.org/10.3923/javaa.2010.1868.1874

SAS Institute Inc SAS/STAT Software (2014). *Version 94 University Edition*. Cary, NC: SAS Institute Inc.

Sauvant, D., Schmidely, P., Daudin, J. J., & St-Pierre, N. R. (2008). Meta-analyses of experimental data in animal nutrition. *Animal*, *2*, 1203–1214. https://doi.org/10.1017/S1751731108002280

Soliva, C. R., Hindrichsen, I. K., Meile, L., Kreuzer, M., & Machmüller, A. (2003). Effects of mixtures of lauric and myristic acid on rumen methanogens and methanogenesis in vitro. *Letters in Applied Microbiology*, *37*, 35–39. https://doi.org/10.1046/j.1472-765X.2003.01343.x

Soliva, C. R., Meile, L., Cieślak, A., Kreuzer, M., & Machmüller, A. (2004). Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. *British Journal of Nutrition*, *92*, 689–700. https://doi.org/10.1079/bjn20041250

Staerfl, S. M., Kreuzer, M., & Soliva, C. R. (2010). In vitro screening of unconventional feeds and various natural supplements for their ruminai methane mitigation potential when included in a maize-silage based diet. *Journal of Animal and Feed Sciences*, *19*, 651–664.

St-Pierre, N. R. (2001). Invited review. Integrating quantitative findings from multiple studies using mixed model methodology. *Journal of Dairy Science*, *84*, 741–755. https://doi.org/10.3168/jds.S0022-0302(01)74530-4

Suryani, H., Zain, M., Ningrat, R. W. S., & Jamarun, N. (2017). Effect of dietary supplementation based on an ammoniated palm frond with direct fed microbials and virgin coconut oil on the growth performance and methane production of bali cattle. *Pakistan Journal of Nutrition*, *16*, 599–604. https://doi.org/10.3923/pjn.2017.599.604

Szczechowiak, J., Szkudelska, K., Szumacher-Strabel, M., Sadkowski, S., Gwozdz, K., El-Sherbiny, M., … Cieslak, A. (2018). Blood hormones, metabolic parameters and fatty acid proportion in dairy cows fed condensed tannins and oils blend. *Annals of Animal Science*, *18*, 155–166. https://doi.org/10.1515/aoas-2017-0039

Ushida, K., Umeda, M., Kishigami, N., & Kojima, Y. (1992). Effect fatty of medium-chain acid calcium and salts and on rumen microorganisms digestion fiber in sheep. *Journal of Animal Science and Technology*, *63*, 591–597.

Van Zijderveld, S. M., Fonken, B., Dijkstra, J., Gerrits, W. J. J., Perdok, H. B., Fokkink, W., & Newbold, J. R. (2011). Effects of a combination of feed additives on methane production, diet digestibility, and animal performance in lactating dairy cows. *Journal of Dairy Science*, *94*, 1445–1454. https://doi.org/10.3168/jds.2010-3635

Varadyova, Z., Certik, M., & Jalc, D. (2018). The possible application of fungal enriched substrates in ruminant nutrition. A review. *Journal of Animal and Feed Sciences*, *27*, 3–10. https://doi.org/10.22358/jafs/84787/2018

Vargas, J. E., Andrés, S., Snelling, T. J., López-Ferreras, L., Yáñez-Ruíz, D. R., García-Estrada, C., & López, S. (2017). Effect of Sunflower and Marine Oils on Ruminal Microbiota, In vitro Fermentation and Digesta Fatty Acid Profile. *Frontiers in Microbiology*, *8*, 1124. https://doi.org/10.3389/fmicb.2017.01124

Wang, K., Nan, X., Chu, K., Tong, J., Yang, L., Zheng, S., … Xiong, B. (2018). Shifts of Hydrogen Metabolism From Methanogenesis to Propionate Production in Response to Replacement of Forage Fiber With Non-forage Fiber Sources in Diets in vitro. *Frontier Microbiology*, *9*, 2764. https://doi.org/10.3389/fmicb.2018.02764

Wang, S., Kreuzer, M., Braunb, U., & Schwarma, A. (2017). Effect of unconventional oilseeds (safflower, poppy, hemp, camelina) on in vitro ruminal methane production and fermentation. *Journal of the Science of Food and Agriculture*, *97*, 3864–3870. https://doi.org/10.1002/jsfa.8260

Yabuuchi, Y., Matsushita, Y., Otsuka, H., Fukamachi, K., & Kobayashi, Y. (2006). Effects of supplemental lauric acid-rich oils in high-grain diet on in vitro rumen fermentation. *Journal of Animal Science*, *77*, 300–307. https://doi.org/10.1111/j.1740-0929.2006.00352.x

Zhang, Z. W., Cao, Z. J., Wang, Y. L., Wang, Y. J., Yang, H. J., & Li, S. L. (2018). Nitro compounds as potential methanogenic inhibitors in ruminant animals: A review. *Animal Feed Science and Technology*, *236*, 107–114. https://doi.org/10.1016/j.anifeedsci.2017.12.010

How to cite this article: Yanza YR, Szumacher-Strabel M, Jayanegara A, et al. The effects of dietary medium-chain fatty acids on ruminal methanogenesis and fermentation in vitro and in vivo: A meta-analysis. *J Anim Physiol Anim Nutr*. 2020;00:1–16. https://doi.org/10.1111/jpn.13367