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

Y. R. Yanza, M. Szumacher-Strabel, **A. Jayanegara, A.M. Kasenta,** M. Gao, H. Huang, A. K. Patra, E. Warzych, A. Cieślak

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Animal and Feed Nutrition Modeling Research Group
IPB University, Bogor

Email: *yulianri.yanza@up.poznan.pl* ; yanza.pulspoznan@gmail.com

Aim

- Thinking the right topic and aim
- Verified that our topic and aim that we thought were already published by previous researchers. If yes, then collecting all related journals to ours.

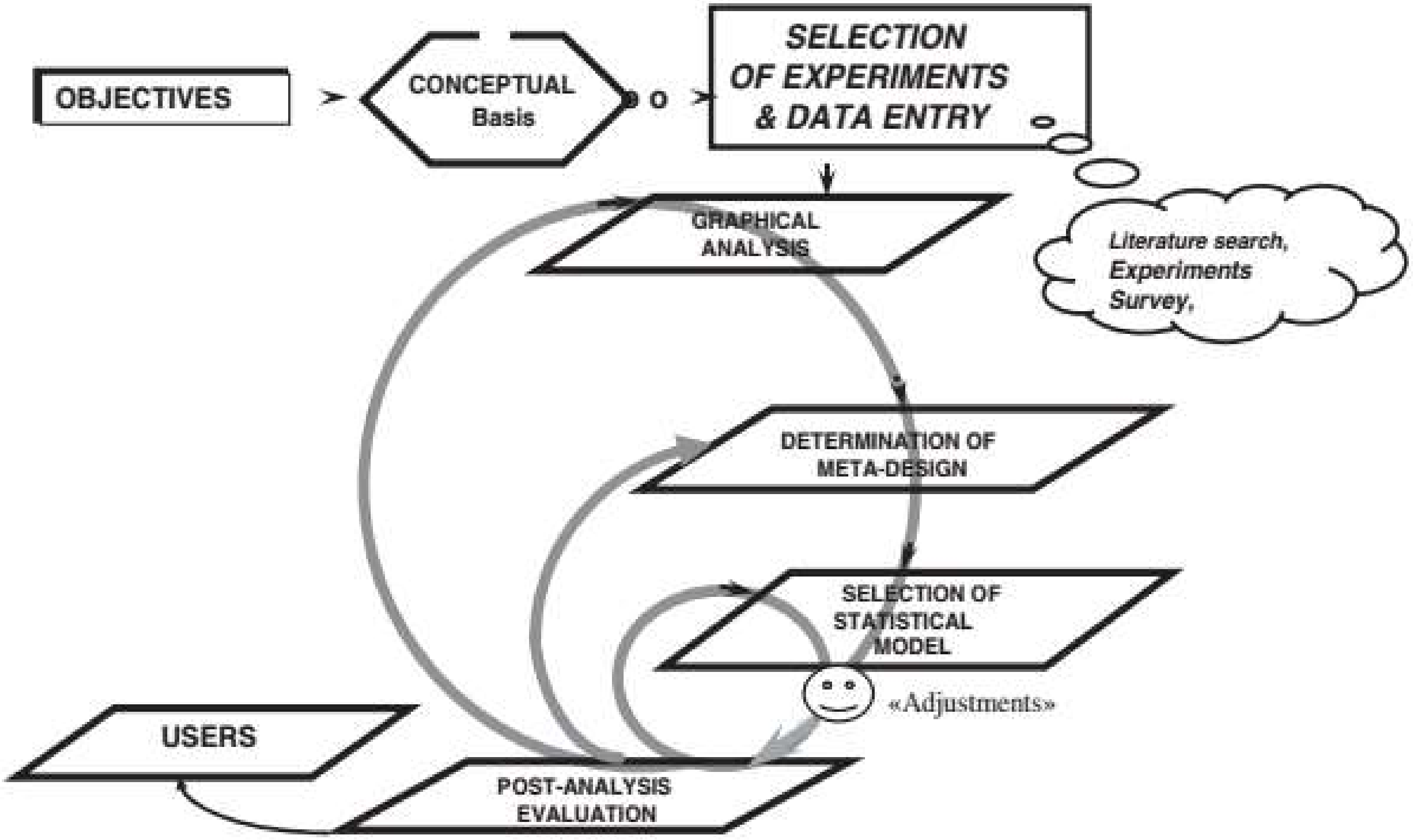
Data

- Input all data of different journals into a database into the same sheet
- Verify our database units, must be similar!
- The statistical power is based on sample size.

Analysis and Interpretation

- Choosing the statistical engine. SAS, R, Stata, Comprehensive Meta-analysis, SPSS, Phyton
- SAS proc :PROC MIXED, GLM, class: study becomes random factor, R: mixed effect model
- Figures, tables, and results interpretation: linear or quadratic response, single or multivariate models, continuous or discrete model

Methods



**Sauvant et al (2008):* Animal, 2:8, 1203–1214

Hypothesis

General idea concept:

Independent variable

Measured variable :
Dosage, diet, etc.
(var. X)

Object measurement

Person, animal, plant, building, cell, etc.

Dependent variable

Parameter (height, intake, size, etc.
(var. Y)

The effect of MCFA dietary on rumen fermentation

Hypothesis

General idea concept:

MCFA

SOURCE

Inclusion in the ruminant diet and verified *in vitro* and *in vivo*

Ruminant

metabolism

Microbial and rumen fermentation

Methane

MITIGATION

Mitigate rumen methane emission without negative impact on ruminant production

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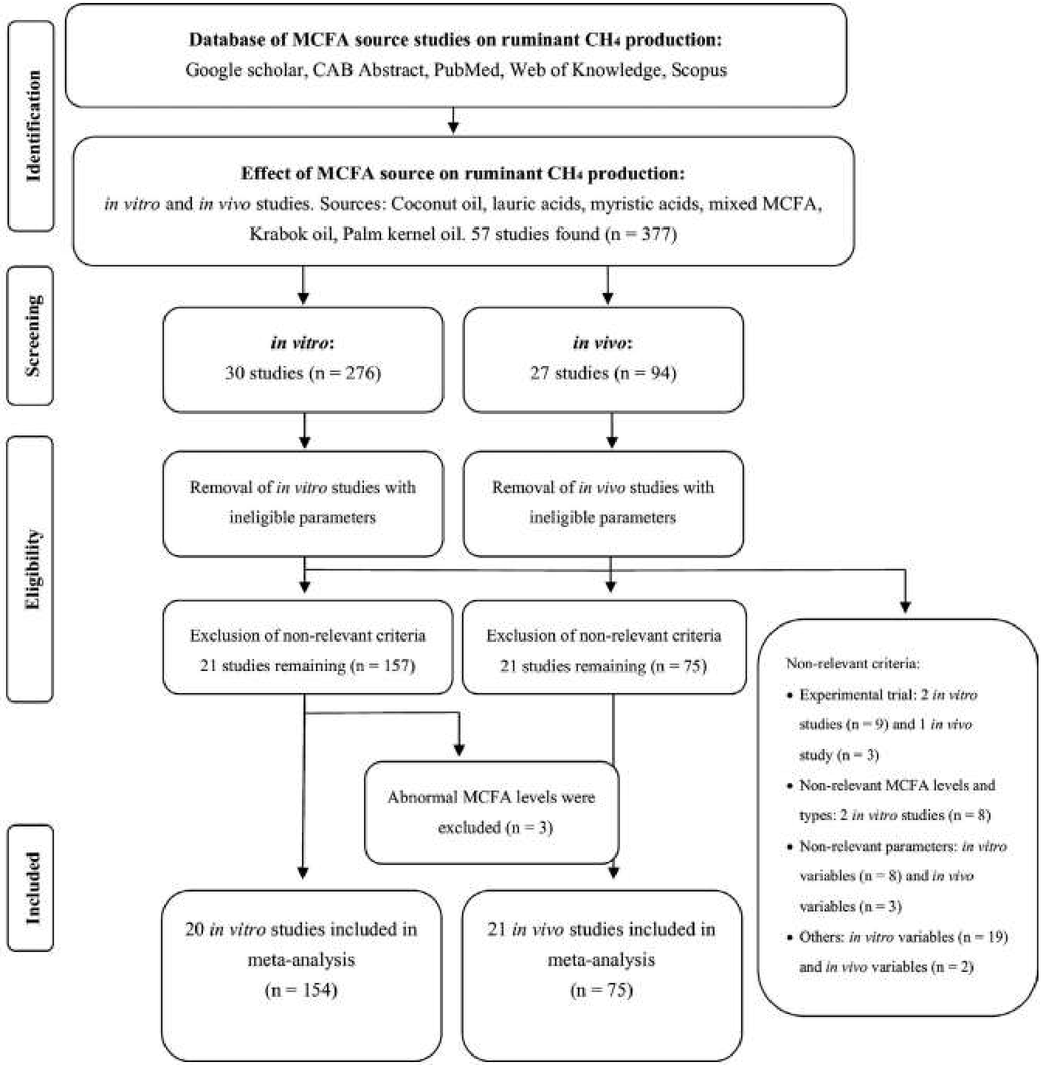

FIGURE 1 Flow diagram for selection of the studies in the meta-analysis to investigate the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis in the in vitro and in vivo studies

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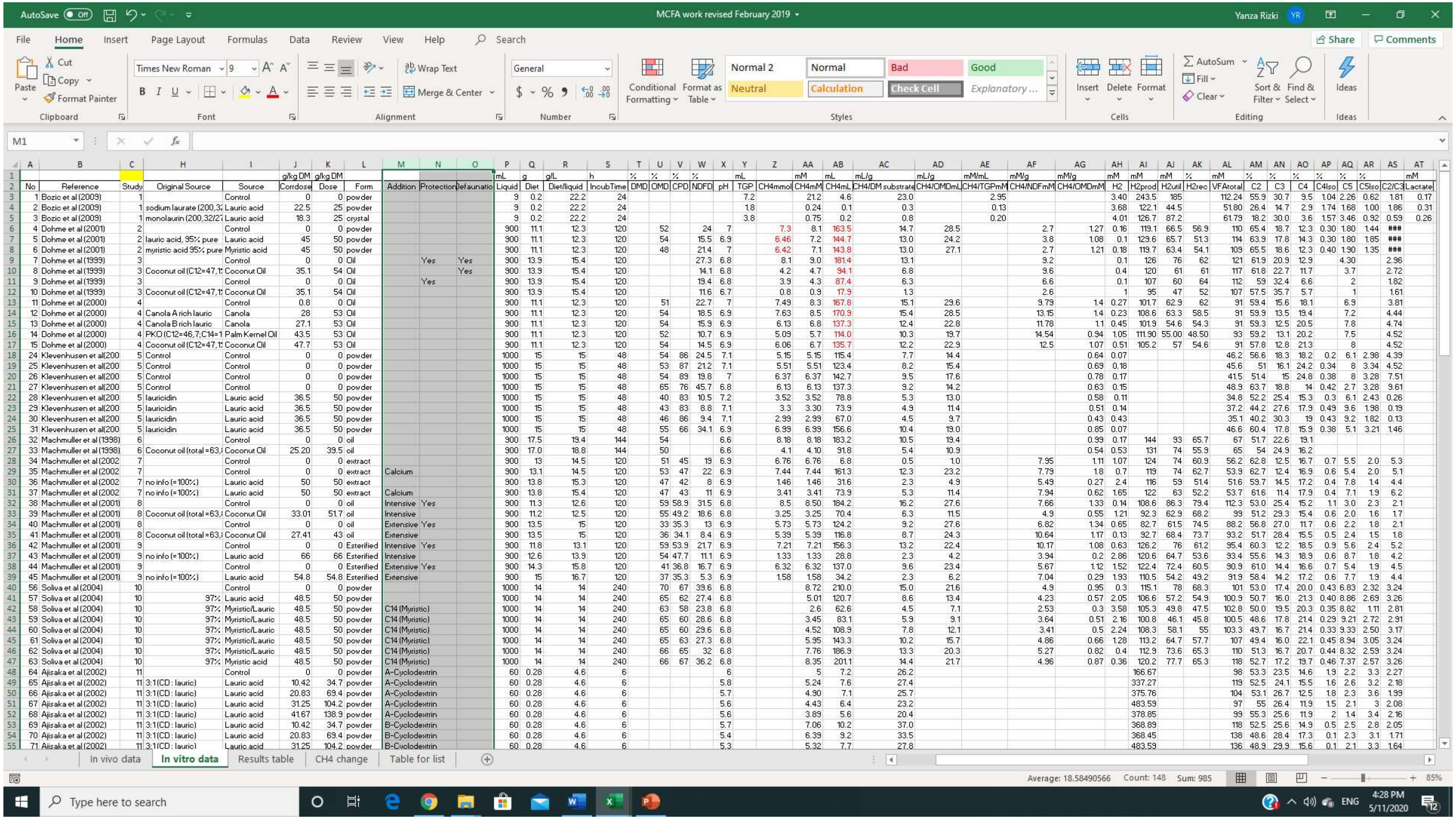

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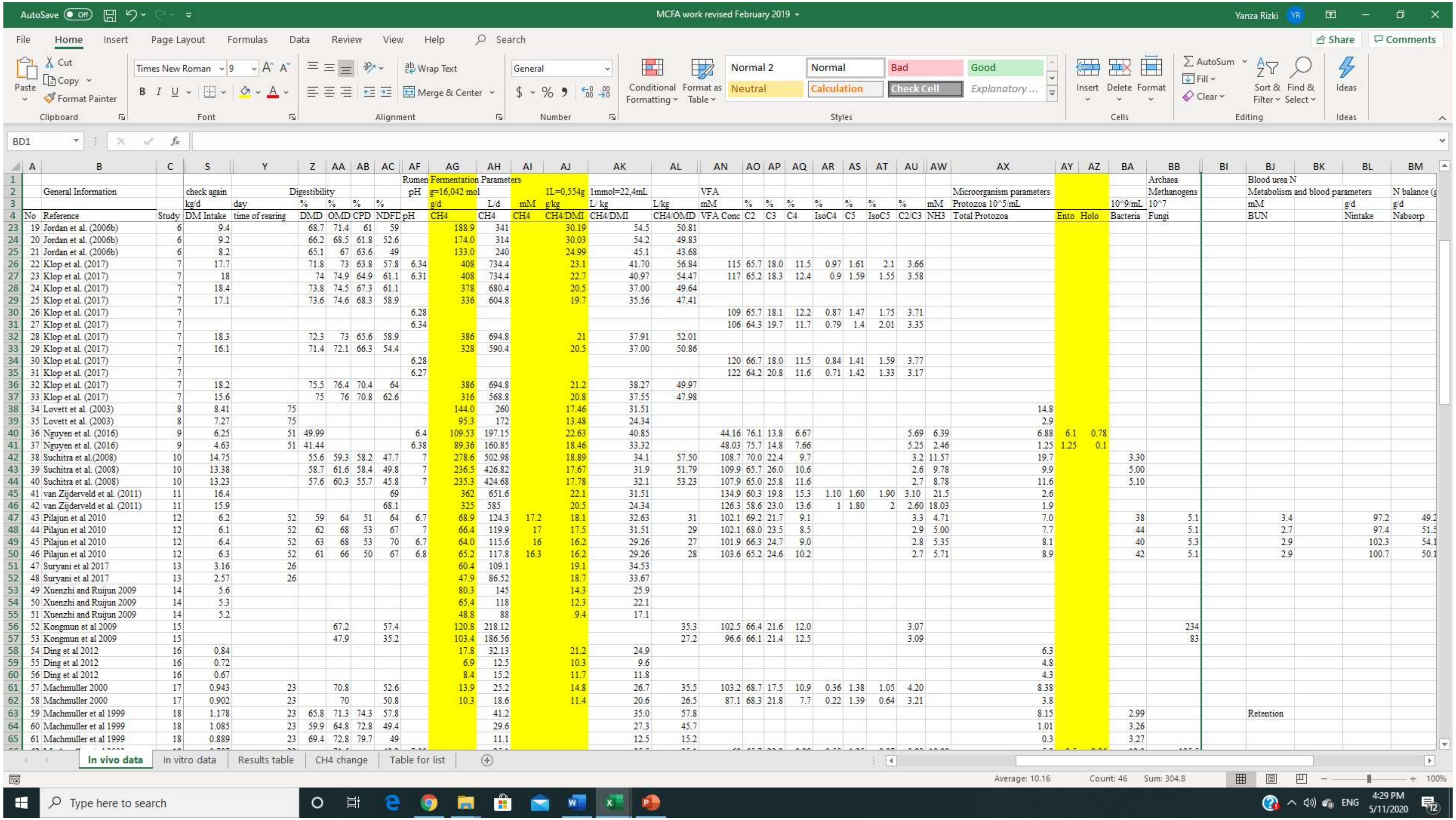

Row	A	B	C	S	Y	Z	AA	AB	AC	AF	AG	AH	AI	AJ
1										Rumen	Fermentation Parameters			
2		General Information		check again		Digestibility				pH	g=16,042 mol			1L=0,554g
3				kg/d	day	%	%	%	%		g/d	L/d	mM	g/kg
4	No	Reference	Study	DM Intake	time of rearing	DMD	OMD	CPD	NDFD	pH	CH4	CH4	CH4	CH4/DMI
23	19	Jordan et al. (2006b)	6	9.4		68.7	71.4	61	59		188.9	341		30.19
24	20	Jordan et al. (2006b)	6	9.2		66.2	68.5	61.8	52.6		174.0	314		30.03
25	21	Jordan et al. (2006b)	6	8.2		65.1	67	63.6	49		133.0	240		24.99
26	22	Klop et al. (2017)	7	17.7		71.8	73	63.8	57.8	6.34	408	734.4		23.1
27	23	Klop et al. (2017)	7	18		74	74.9	64.9	61.1	6.31	408	734.4		22.7
28	24	Klop et al. (2017)	7	18.4		73.8	74.5	67.3	61.1		378	680.4		20.5
29	25	Klop et al. (2017)	7	17.1		73.6	74.6	68.3	58.9		336	604.8		19.7
30	26	Klop et al. (2017)	7							6.28				
31	27	Klop et al. (2017)	7							6.34				
32	28	Klop et al. (2017)	7	18.3		72.3	73	65.6	58.9		386	694.8		21
33	29	Klop et al. (2017)	7	16.1		71.4	72.1	66.3	54.4		328	590.4		20.5
34	30	Klop et al. (2017)	7							6.28				
35	31	Klop et al. (2017)	7							6.27				
36	32	Klop et al. (2017)	7	18.2		75.5	76.4	70.4	64		386	694.8		21.2
37	33	Klop et al. (2017)	7	15.6		75	76	70.8	62.6		316	568.8		20.8
38	34	Lovett et al. (2003)	8	8.41	75						144.0	260		17.46
39	35	Lovett et al. (2003)	8	7.27	75						95.3	172		13.48
40	36	Nguyen et al. (2016)	9	6.25	51	49.99				6.4	109.53	197.15		22.63
41	37	Nguyen et al. (2016)	9	4.63	51	41.44				6.38	89.36	160.85		18.46
42	38	Suchitra et al.(2008)	10	14.75		55.6	59.3	58.2	47.7	7	278.6	502.98		18.89
43	39	Suchitra et al. (2008)	10	13.38		58.7	61.6	58.4	49.8	7	236.5	426.82		17.67
44	40	Suchitra et al. (2008)	10	13.23		57.6	60.3	55.7	45.8	7	235.3	424.68		17.78
45	41	van Zijderveld et al. (2011)	11	16.4					69		362	651.6		22.1
46	42	van Zijderveld et al. (2011)	11	15.9					68.1		325	585		20.5
47	43	Pilajun et al 2010	12	6.2	52	59	64	51	64	6.7	68.9	124.3	17.2	18.1
48	44	Pilajun et al 2010	12	6.1	52	62	68	53	67	7	66.4	119.9	17	17.5
49	45	Pilajun et al 2010	12	6.4	52	63	68	53	70	6.7	64.0	115.6	16	16.2
50	46	Pilajun et al 2010	12	6.3	52	61	66	50	67	6.8	65.2	117.8	16.3	16.2
51	47	Suryani et al 2017	13	3.16	26						60.4	109.1		19.1
52	48	Suryani et al 2017	13	2.57	26						47.9	86.52		18.7
53	49	Xuenzhi and Ruijun 2009	14	5.6							80.3	145		14.3
54	50	Xuenzhi and Ruijun 2009	14	5.3							65.4	118		12.3
55	51	Xuenzhi and Ruijun 2009	14	5.2							48.8	88		9.4
56	52	Kongmun et al 2009	15				67.2		57.4		120.8	218.12		
57	53	Kongmun et al 2009	15				47.9		35.2		103.4	186.56		
58	54	Ding et al 2012	16	0.84							17.8	32.13		21.2
59	55	Ding et al 2012	16	0.72							6.9	12.5		10.3
60	56	Ding et al 2012	16	0.67							8.4	15.2		11.7
61	57	Machmuller 2000	17	0.943	23		70.8		52.6		13.9	25.2		14.8
62	58	Machmuller 2000	17	0.902	23		70		50.8		10.3	18.6		11.4
63	59	Machmuller et al 1999	18	1.178	23	65.8	71.3	74.3	57.8			41.2		
64	60	Machmuller et al 1999	18	1.085	23	59.9	64.8	72.8	49.4			29.6		
65	61	Machmuller et al 1999	18	0.889	23	69.4	72.8	79.7	49			11.1		

Row	AK	AL	AN	AO	AP	AQ	AR	AS	AT	AU	AW	AX	AY	AZ	BA	BB	BI	BJ	BK	BL	BM
1																Archaea		Blood urea N			
2	1mmol=22,4mL		VFA									Microorganism parameters				Methanogens		Metabolism and blood parameters			N balance (g
3	L/ kg	L/kg	mM	%	%	%	%	%	%	%	mM	Protozoa 10^5/mL			10^9/mL	10^7		mM		g/d	g/d
4	CH4/DMI	CH4/OMD	VFA Conc	C2	C3	C4	IsoC4	C5	IsoC5	C2/C3	NH3	Total Protozoa	Ento	Holo	Bacteria	Fungi		BUN		Nintake	Nabsorp
23	54.5	50.81																			
24	54.2	49.83																			
25	45.1	43.68																			
26	41.70	56.84	115	65.7	18.0	11.5	0.97	1.61	2.1	3.66											
27	40.97	54.47	117	65.2	18.3	12.4	0.9	1.59	1.55	3.58											
28	37.00	49.64																			
29	35.56	47.41																			
30			109	65.7	18.1	12.2	0.87	1.47	1.75	3.71											
31			106	64.3	19.7	11.7	0.79	1.4	2.01	3.35											
32	37.91	52.01																			
33	37.00	50.86																			
34			120	66.7	18.0	11.5	0.84	1.41	1.59	3.77											
35			122	64.2	20.8	11.6	0.71	1.42	1.33	3.17											
36	38.27	49.97																			
37	37.55	47.98																			
38	31.51											14.8									
39	24.34											2.9									
40	40.85		44.16	76.1	13.8	6.67				5.69	6.39	6.88	6.1	0.78							
41	33.32		48.03	75.7	14.8	7.66				5.25	2.46	1.25	1.25	0.1							
42	34.1	57.50	108.7	70.0	22.4	9.7				3.2	11.57	19.7			3.30						
43	31.9	51.79	109.9	65.7	26.0	10.6				2.6	9.78	9.9			5.00						
44	32.1	53.23	107.9	65.0	25.8	11.6				2.7	8.78	11.6			5.10						
45	31.51		134.9	60.3	19.8	15.3	1.10	1.60	1.90	3.10	21.5	2.6									
46	24.34		126.3	58.6	23.0	13.6	1	1.80	2	2.60	18.03	1.9									
47	32.63	31	102.1	69.2	21.7	9.1				3.3	4.71	7.0			38	5.1		3.4		97.2	49.2
48	31.51	29	102.1	68.0	23.5	8.5				2.9	5.00	7.7			44	5.1		2.7		97.4	51.5
49	29.26	27	101.9	66.3	24.7	9.0				2.8	5.35	8.1			40	5.3		2.9		102.3	54.1
50	29.26	28	103.6	65.2	24.6	10.2				2.7	5.71	8.9			42	5.1		2.9		100.7	50.1
51	34.53																				
52	33.67																				
53	25.9																				
54	22.1																				
55	17.1																				
56		35.3	102.5	66.4	21.6	12.0				3.07						234					
57		27.2	96.6	66.1	21.4	12.5				3.09						83					
58	24.9											6.3									
59	9.6											4.8									
60	11.8											4.3									
61	26.7	35.5	103.2	68.7	17.5	10.9	0.36	1.38	1.05	4.20		8.38									
62	20.6	26.5	87.1	68.3	21.8	7.7	0.22	1.39	0.64	3.21		3.8									
63	35.0	57.8										8.15			2.99			Retention			
64	27.3	45.7										1.01			3.26						
65	12.5	15.2										0.3			3.27						

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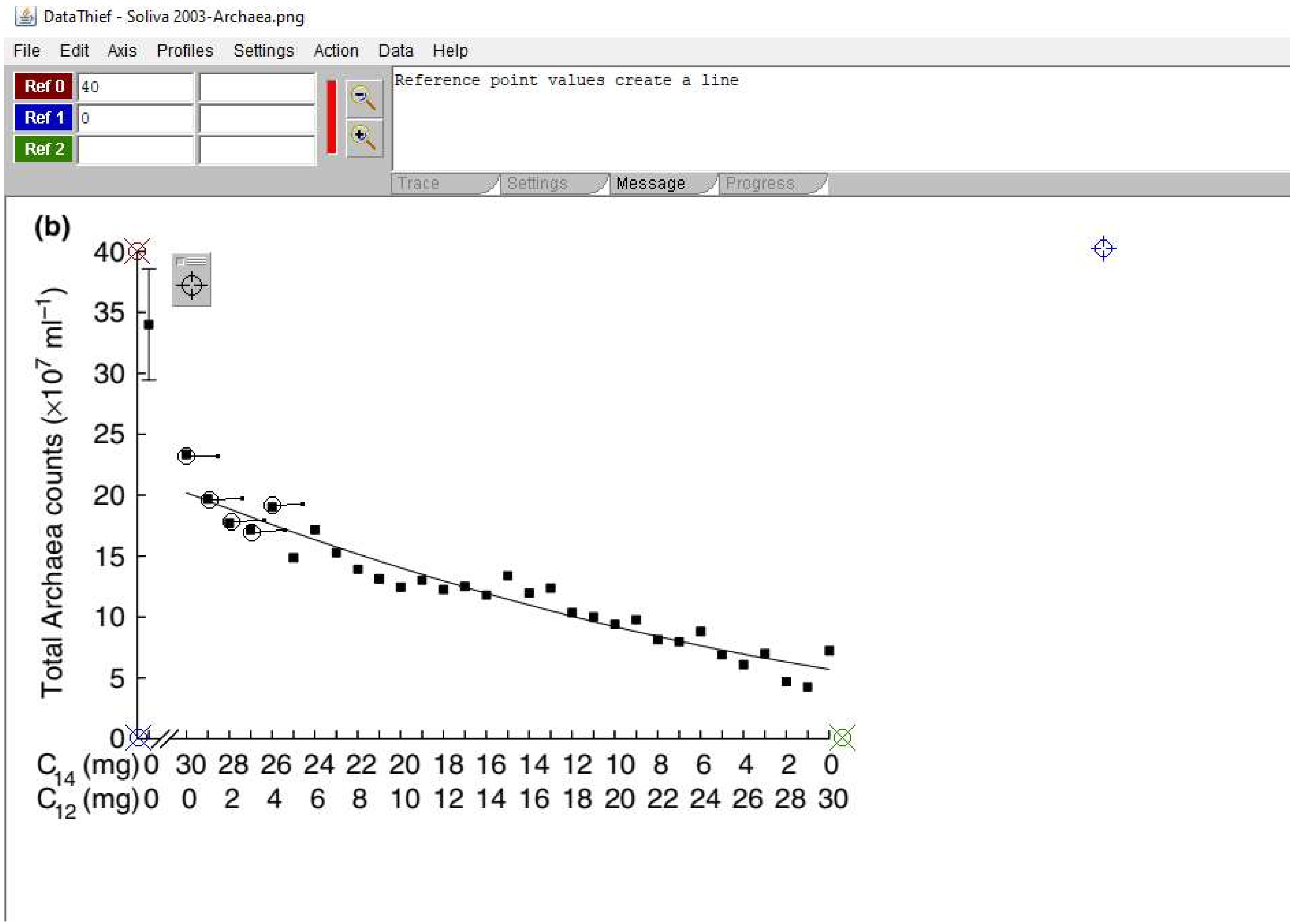

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TABLE 1 In vitro experiments included in the meta-analysis of the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis

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	
8	Machmüller, Soliva, and Kreuzer (2002)	RUSITEC	Hay, corn silage, concentrates	LA	
9	Ajisaka et al. (2002)	GVI	Glucose, cellobiose	LA	
10	Soliva et al. (2003)	HGT	Not specified	LA, MA, LMA	
11	Soliva et al. (2004)	RUSITEC	Grass hay, concentrate	LA, MA, LMA	
12	Yabuuchi et al. (2006)	GBI	Corn grains	PKO, CO	
13	Cieślak et al. (2006)	RUSITEC	Hay; wheat meal	CO	
14	Božic et al. (2009)	CBC	Alfalfa	LA	
15	Klevenhusen et al. (2009)	RUSITEC	Maize, wheat; hay	LA	
16	Staerfl, Kreuzer, and Soliva (2010)	HGT	Maize silage, concentrate	LA, MA	
17	O'brien, Navarro-Villa, Purcell, Boland, and O'Kiely (2014)	GPT	Ryegrass, grass silage, barley grains	LA	
18	Kim et al. (2014)	GVI	Alfalfa; concentrate	LA	
19	Kang et al. (2016)	GBI	Cassava chips, rice bran; coconut and palm meals	KO, CO	
20	Kang et al. (2017)	GBI	Rice straw, cassava	KO	

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

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.

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variables. The continuous predictor variable consisted of the concentrations of MCFA supplementation. The statistical model used was (Equation 1):

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

Regression

Means comparison

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

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

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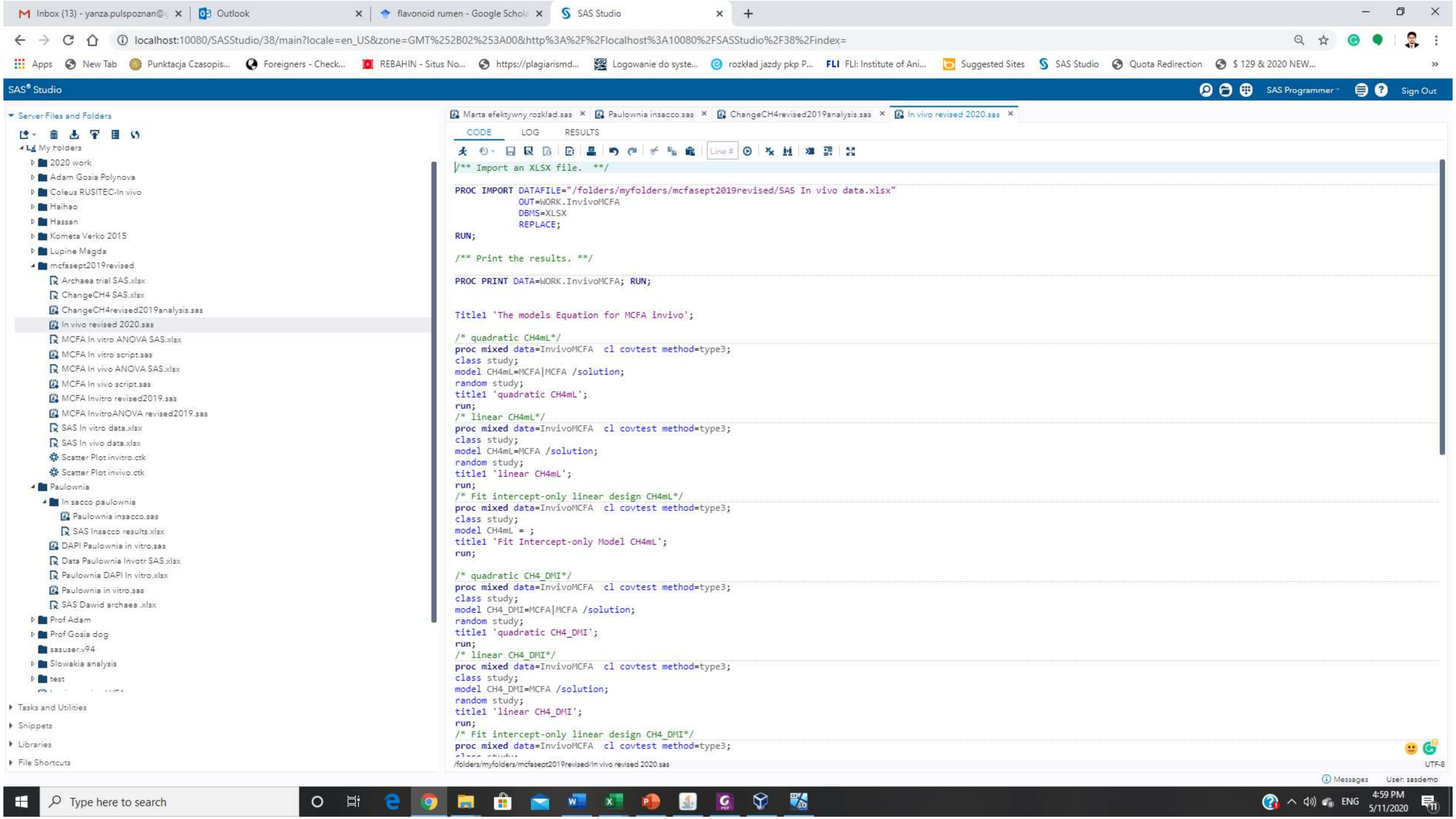

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quadratic CH4mL

The Mixed Procedure

Model Information	
Data Set	WORK.INVIVOMCFA
Dependent Variable	CH4mL
Covariance Structure	Variance Components
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Study	21	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Dimensions	
Covariance Parameters	2
Columns in X	3
Columns in Z	21
Subjects	1
Max Obs per Subject	60

Number of Observations	
Number of Observations Read	75
Number of Observations Used	60
Number of Observations Not Used	15

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
MCFA	1	36399	36399	Var(Residual) + Q(MCFA)	MS(Residual)	41	5.72	0.0215
MCFA*MCFA	1	12718	12718	Var(Residual) + Q(MCFA*MCFA)	MS(Residual)	41	2.00	0.1651
Study	16	3878081	242380	Var(Residual) + 3.3632 Var(Study)	MS(Residual)	41	38.06	<.0001
Residual	41	261081	6367.840064	Var(Residual)	.	.	.	.

quadratic CH4mL

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
Study	70175	31833	2.20	0.0275	0.05	7784.15	132566
Residual	6367.84	1415.72	4.50	<.0001	0.05	4311.08	10354

Fit Statistics	
-2 Res Log Likelihood	746.3
AIC (Smaller is Better)	750.3
AICC (Smaller is Better)	750.6
BIC (Smaller is Better)	752.4

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	313.21	66.2456	16	4.73	0.0002
MCFA	-3.6232	1.4810	41	-2.45	0.0188
MCFA*MCFA	0.03327	0.02272	41	1.46	0.1507

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
MCFA	1	41	5.99	0.0188
MCFA*MCFA	1	41	2.14	0.1507

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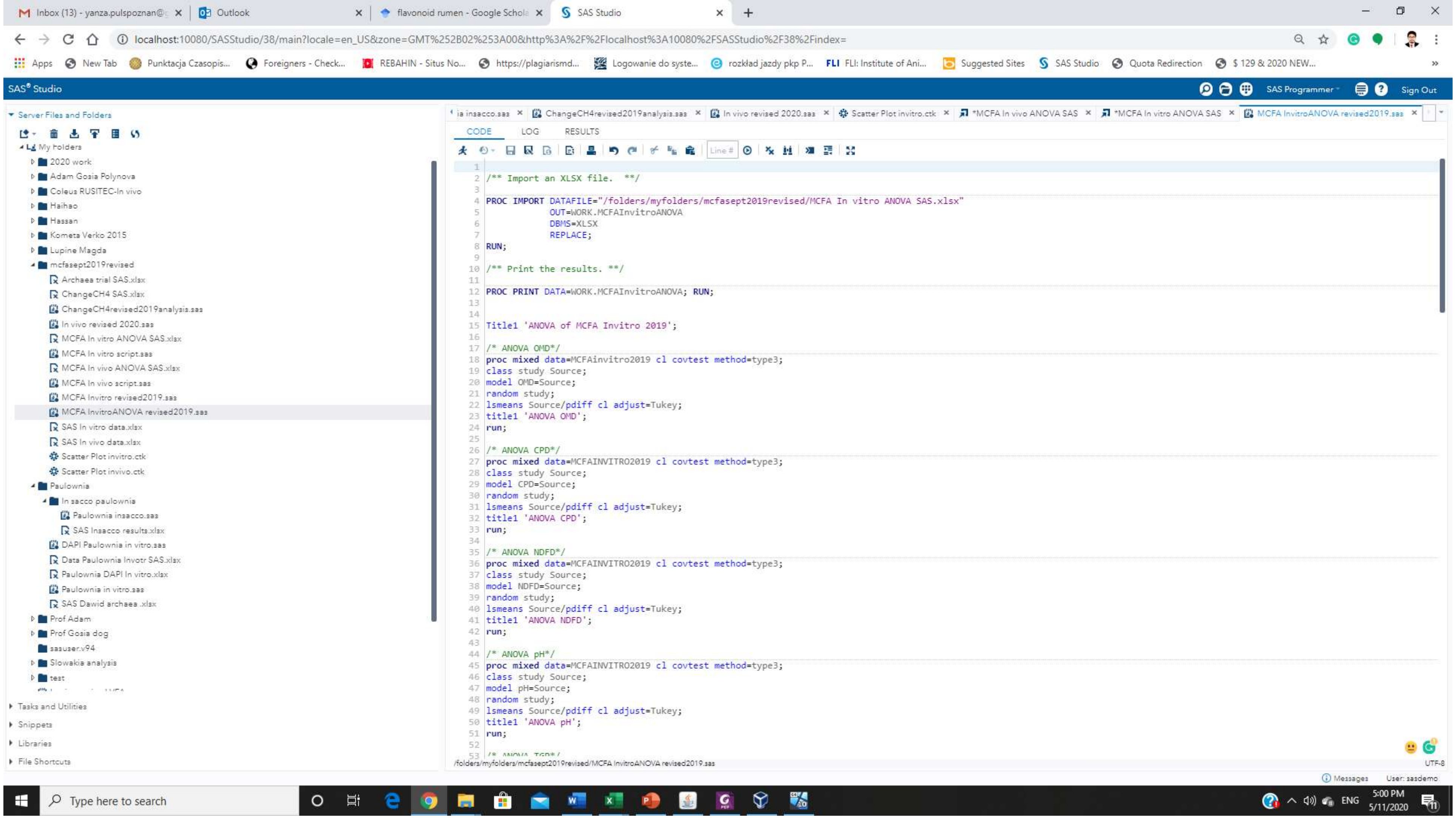

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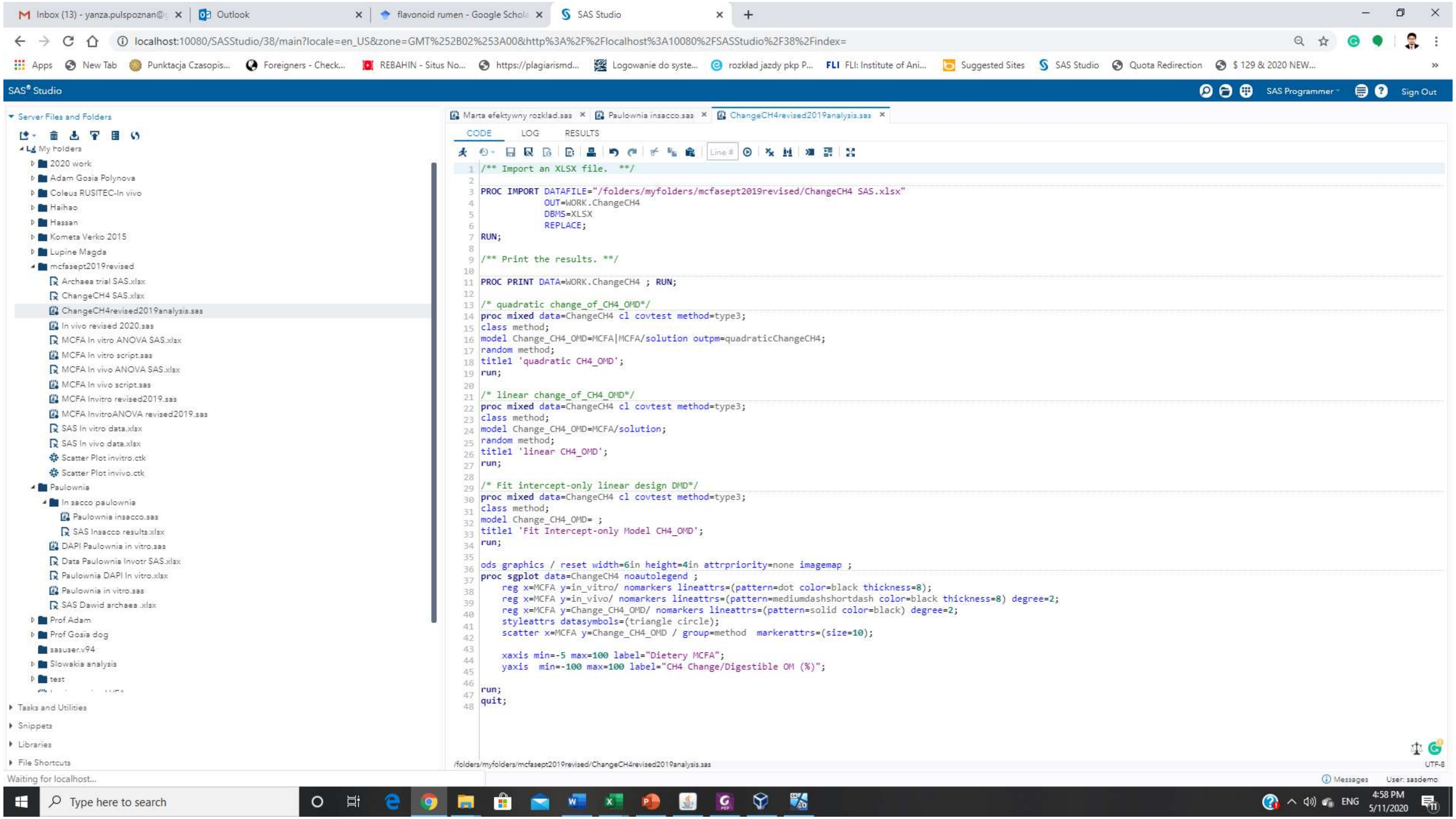

Results

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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.

Results

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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 $p \le .10$. The model is considered compatible at $p \le .05$.

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.

Results

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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 $p \le .05$.

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.

Results

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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 $p \le .10$. The model is considered compatible at $p \le .05$.

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.

Results

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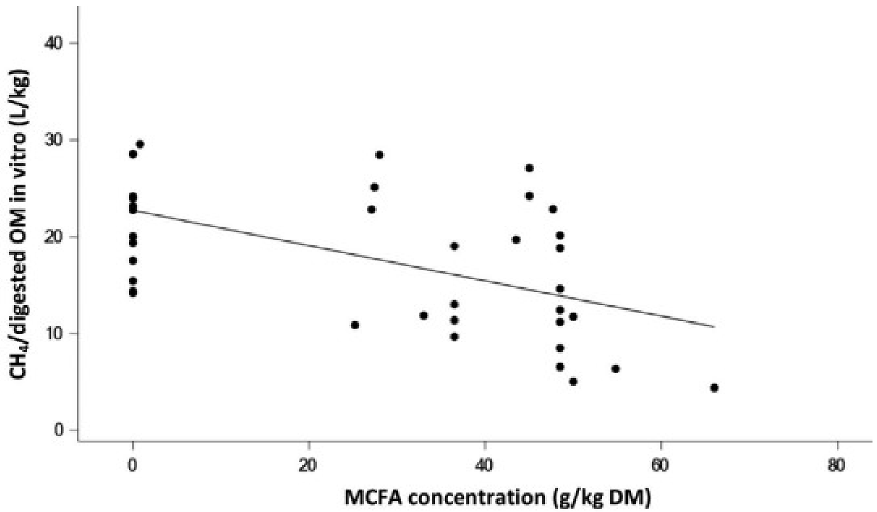
TABLE 7 Responses of ruminal fermentation parameters affected by different medium-chain fatty acids (MCFA) sources in the in vivo experiments

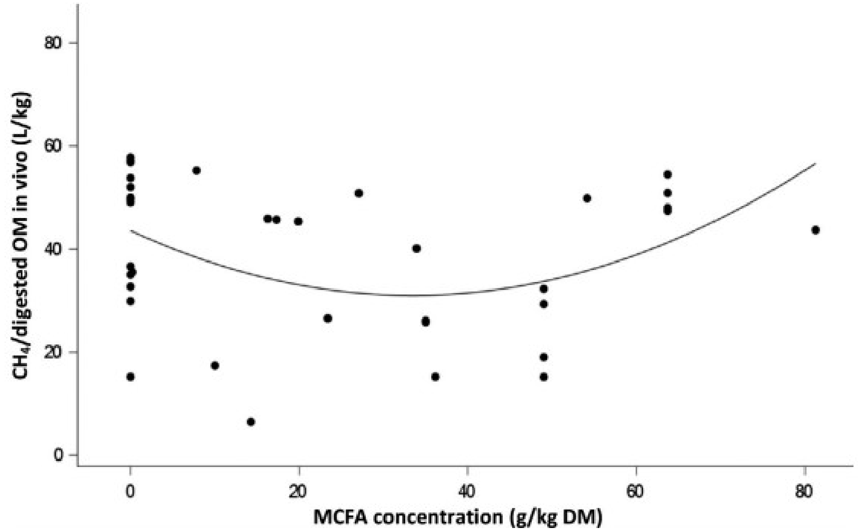
Response parameters	*n*	Control	CO	LA	MA	LMA	*p* value
DMI (kg/d)	65	10.3^a	8.90^b	8.75^b	10.2^{ab}	10.0^{ab}	.01
In vivo digestibility (%)							
DMD	30	67.8^a	64.8^b	69.3^a	–	–	.03
OMD	43	70.1	68.6	71.5	68.0	–	ns
CPD	32	65.8	66.7	66.6	–	–	ns
NDFD	46	57.5	53.0	57.9	53.4	60.4	ns
Rumen fermentation							
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 $p \le .05$.

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.

Results





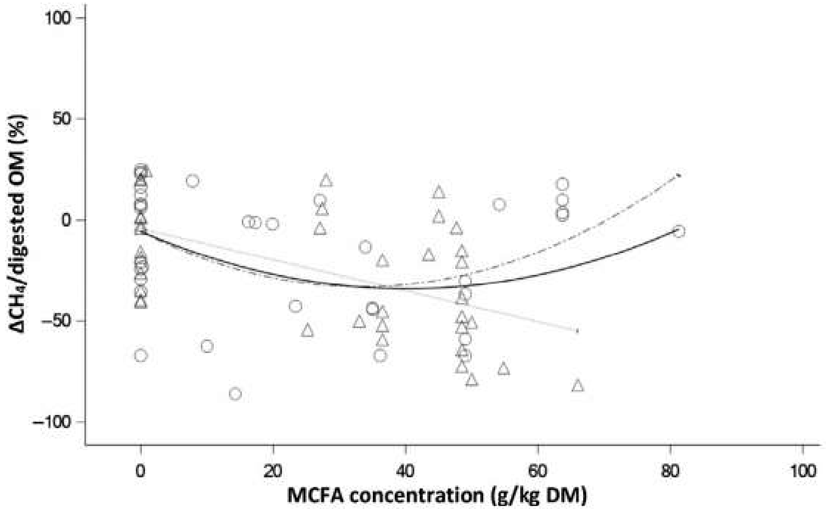


FIGURE 3 Relative changes in methane production with dietary medium-chain fatty acids (MCFA) concentrations in vitro and in vivo. Methane production (L/kg organic matter digested) converted to percentage change was predicted based on MCFA concentration (g/kg DM) in the in vitro (Δ, dot regression line)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


Conclusion

- Increasing the MCFA concentrations caused a decrease in CH4 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 CH4 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.

Example of other project


Thank you
for
Your attentions!
Yuk kerja bareng!