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

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**2<sup>nd</sup> AFENUE online meeting, Tuesday, 12 May 2020**  
**Animal and Feed Nutrition Modeling Research Group**  
**IPB University, Bogor**

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## 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

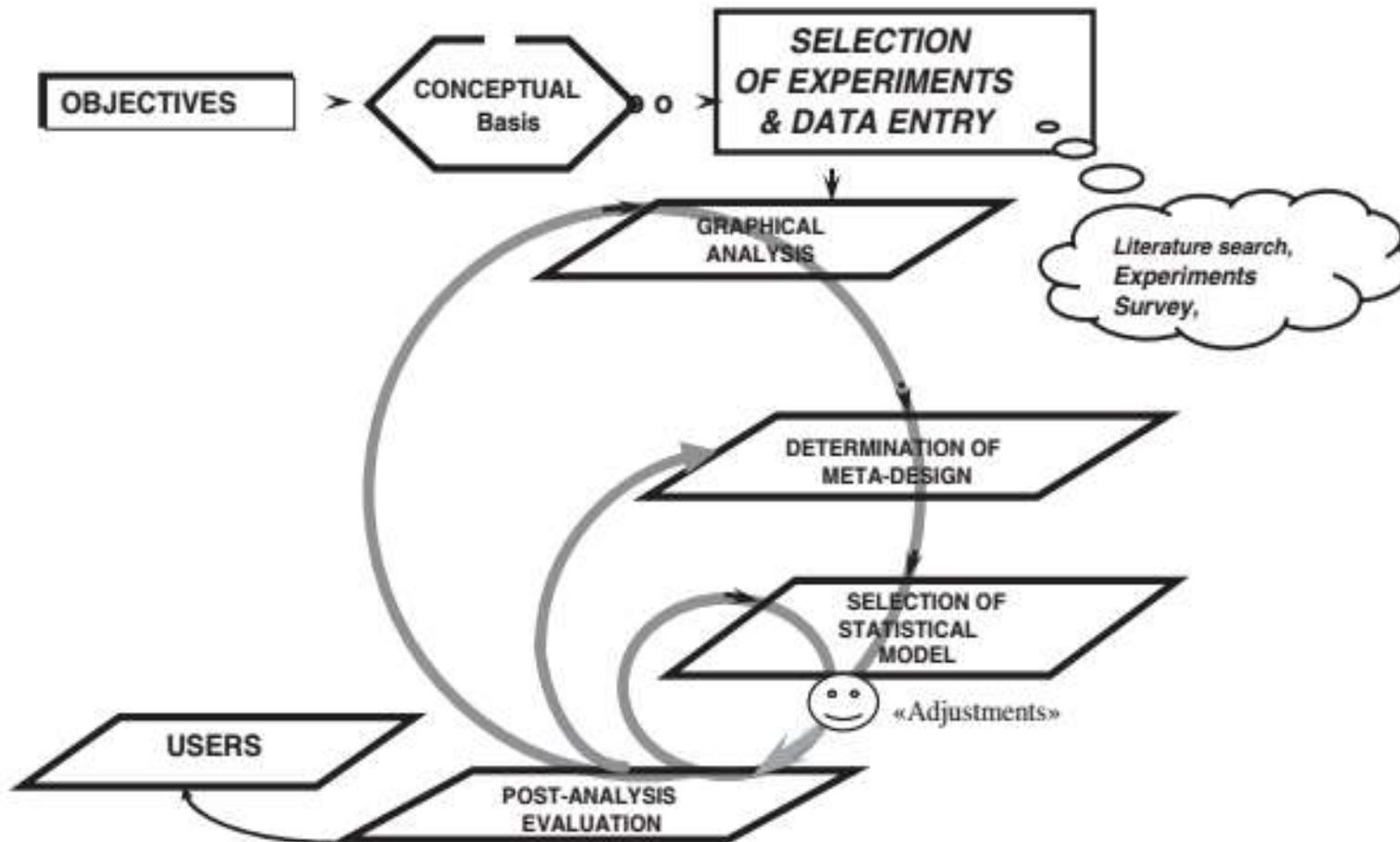
- Choosing the statistical engine. SAS, R, Stata, Comprehensive Meta-analysis, SPSS, Phytion
- SAS proc :PROC MIXED, GLM, class: study becomes random factor, R: mixed effect model
- Figures, tables, and results interpretation: linear or quadratic response, single or multivariate models, continuous or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



# Methods



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

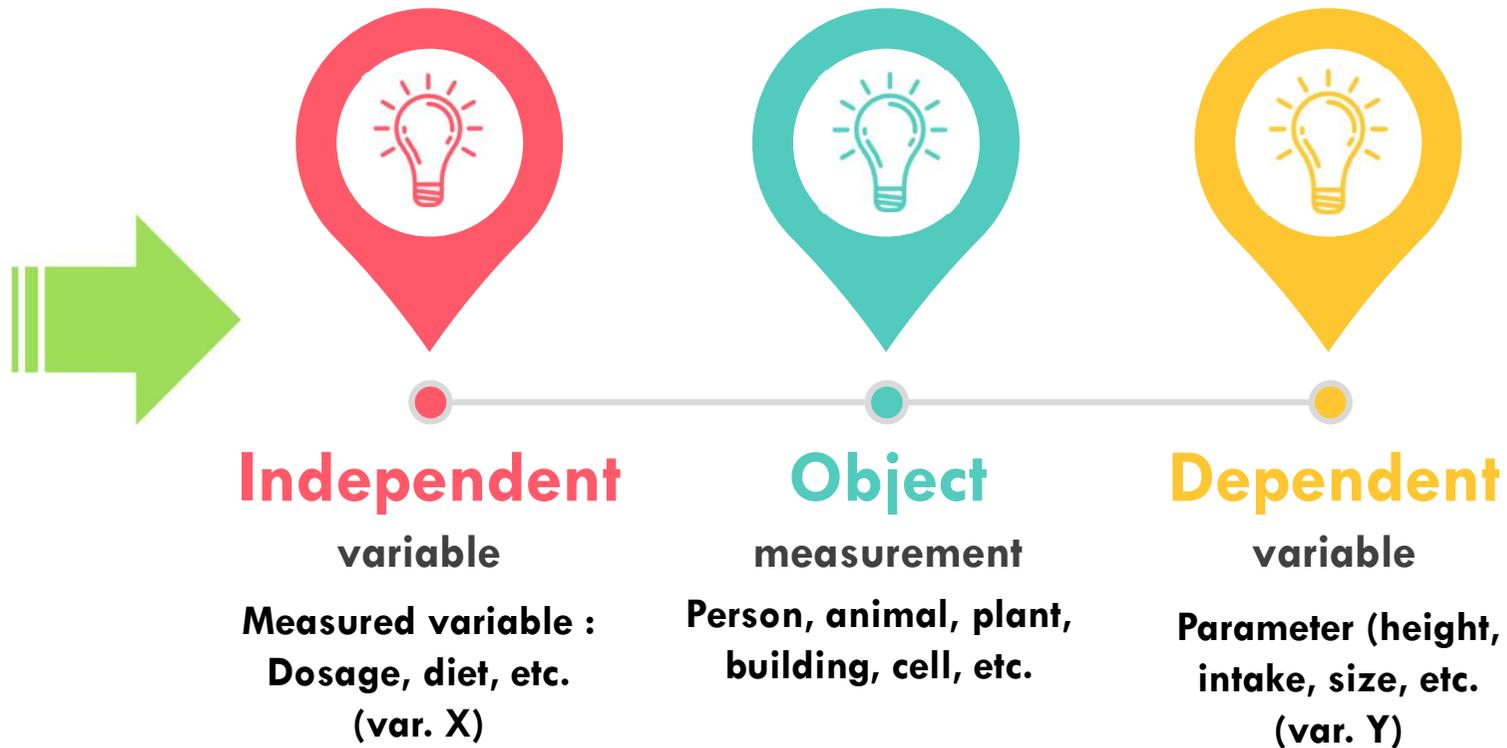


# Hypothesis



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## General idea concept:





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**The effect of  
MCFA dietary on  
rumen  
fermentation**

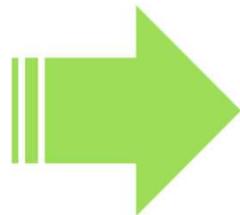


# Hypothesis



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## 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**



# Methods



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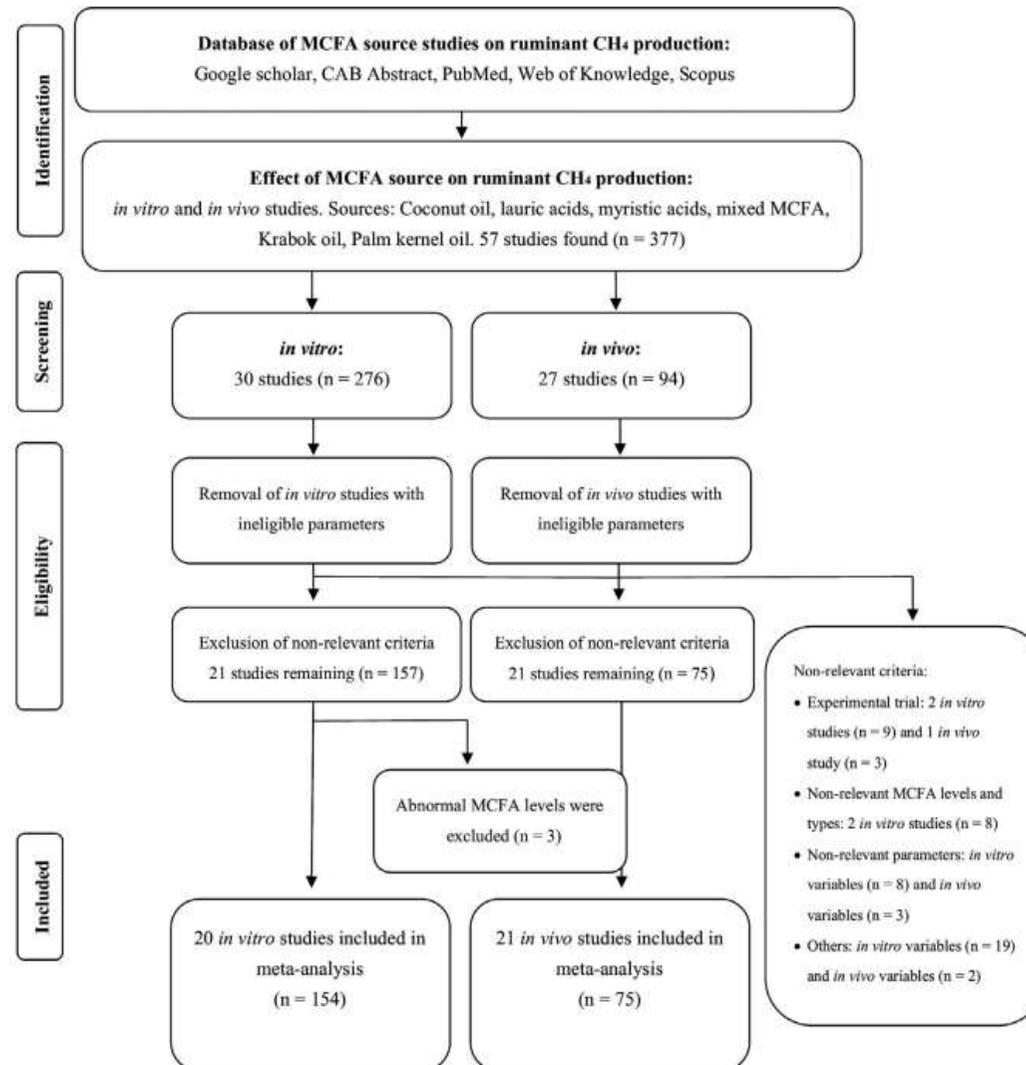


FIGURE 1 Flow diagram for selection of the studies in the meta-analysis to investigate the effect of medium-chain fatty acids (MCFA) on ruminal methanogenesis in the *in vitro* and *in vivo* studies





# Methods



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AutoSave  MCF work revised February 2019 Yanza Rizki

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1	General Information		check again	Digestibility			Rumen Fermentation Parameters										Microorganism parameters																						
2			kg/d	day	%	%	%	pH	pH	pH	pH	L/d	mM	g/kg	IL=0.554g	Immol=22.4mL	VFA	%	%	%	%	%	%	%	mM	Protozoa 10 <sup>5</sup> /mL			Archaea		Blood urea N		Metabolism and blood parameters		N balance (g/d)				
3																	Conc	C2	C3	C4	IsoC4	C5	IsoC5	C2/C3	NH3	Total Protozoa	Ento	Holo	Bacteria	Fungi	10 <sup>9</sup> /mL	10 <sup>7</sup>	mM	BUN	g/d	N intake	Nabsorp		
19	Jordan et al. (2006b)	6	9.4	68.7	71.4	61	59	188.9	341	30.19	54.5	50.81																											
20	Jordan et al. (2006b)	6	9.2	66.2	68.5	61.8	52.6	174.0	314	30.03	54.2	49.83																											
21	Jordan et al. (2006b)	6	8.2	65.1	67	63.6	49	133.0	240	24.99	45.1	43.68																											
22	Klop et al. (2017)	7	17.7	71.8	73	63.8	57.8	6.34	408	734.4	23.1	41.70	56.84	115	65.7	18.0	11.5	0.97	1.61	2.1	3.66																		
23	Klop et al. (2017)	7	18	74	74.9	64.9	61.1	6.31	408	734.4	22.7	40.97	54.47	117	65.2	18.3	12.4	0.9	1.59	1.55	3.58																		
24	Klop et al. (2017)	7	18.4	73.8	74.5	67.3	61.1		378	680.4	20.5	37.00	49.64																										
25	Klop et al. (2017)	7	17.1	73.6	74.6	68.3	58.9		336	604.8	19.7	35.56	47.41																										
26	Klop et al. (2017)	7						6.28									109	65.7	18.1	12.2	0.87	1.47	1.75	3.71															
27	Klop et al. (2017)	7						6.34									106	64.3	19.7	11.7	0.79	1.4	2.01	3.55															
28	Klop et al. (2017)	7	18.3	72.3	73	65.6	58.9		386	694.8	21	37.91	52.01																										
29	Klop et al. (2017)	7	16.1	71.4	72.1	66.3	54.4		328	590.4	20.5	37.00	50.86																										
30	Klop et al. (2017)	7						6.28									120	66.7	18.0	11.5	0.84	1.41	1.59	3.77															
31	Klop et al. (2017)	7						6.27									122	64.2	20.8	11.6	0.71	1.42	1.33	3.17															
32	Klop et al. (2017)	7	18.2	75.5	76.4	70.4	64		386	694.8	21.2	38.27	49.97																										
33	Klop et al. (2017)	7	13.6	75	76	70.8	62.6		316	568.8	20.8	37.55	47.98																										
34	Lovett et al. (2003)	8	8.41	75				144.0	0	260	17.46	31.51														14.8													
35	Lovett et al. (2003)	8	7.27	75				95.3	172		13.48	24.34														2.9													
36	Nguyen et al. (2016)	9	6.25	51	49.99			109.53	197.15	22.63	40.85			44.16	76.1	13.8	6.67						5.69	6.39	6.88	6.1	0.78												
37	Nguyen et al. (2016)	9	4.63	51	41.44			89.36	160.85	18.46	33.32			48.03	75.7	14.8	7.66						5.25	2.46	1.25	1.25	0.1												
38	Suchitra et al. (2008)	10	14.75	55.6	59.3	58.2	47.7	7	278.6	502.98	18.89	34.1	57.50	108.7	70.0	22.4	9.7							3.2	11.57	19.7			3.30										
39	Suchitra et al. (2008)	10	13.38	58.7	61.6	58.4	49.8	7	236.5	426.82	17.67	31.9	51.79	109.9	65.7	26.0	10.6							2.6	9.78	9.9			5.00										
40	Suchitra et al. (2008)	10	13.23	57.6	60.3	55.7	45.8	7	235.3	424.68	17.78	32.1	53.23	107.9	65.0	25.8	11.6							2.7	8.78	11.6			5.10										
41	van Zijderveld et al. (2011)	11	16.4					362	651.6	22.1	31.51			134.9	60.3	19.8	15.3			1.10	1.60	1.90	3.10	21.5	2.6														
42	van Zijderveld et al. (2011)	11	15.9					325	585	20.5	24.34			126.3	58.6	23.0	13.6			1	1.80	2	2.60	18.03	1.9														
43	Pilajun et al. 2010	12	6.2	52	59	64	51	64	6.7	68.9	124.3	17.2	18.1	32.63	31	102.1	69.2	21.7	9.1				3.3	4.71	7.0														
44	Pilajun et al. 2010	12	6.1	52	62	68	53	67	7	66.4	119.9	17	17.5	31.51	29	102.1	68.0	23.5	8.5				2.9	5.00	7.7				38		5.1			3.4		97.2	49.2		
45	Pilajun et al. 2010	12	6.4	52	63	68	53	70	6.7	64.0	115.6	16	16.2	29.26	27	101.9	66.3	24.7	9.0				2.8	5.55	8.1				44		5.1			2.7		5.00	51.5		
46	Pilajun et al. 2010	12	6.3	52	61	66	50	67	6.8	65.2	117.8	16.3	16.2	29.26	28	103.6	65.2	24.6	10.2				2.7	5.71	8.9				40		5.3			2.9		102.3	54.1		
47	Suryani et al. 2017	13	3.16	26				60.4	109.1	19.1	34.53																												
48	Suryani et al. 2017	13	2.57	26				47.9	86.52	18.7	33.67																												
49	Xuenzhi and Ruijun 2009	14	5.6					80.3	145	14.3	25.9																												
50	Xuenzhi and Ruijun 2009	14	5.3					65.4	118	12.3	22.1																												
51	Xuenzhi and Ruijun 2009	14	5.2					48.8	88	9.4	17.1																												
52	Kongmun et al. 2009	15			67.2		57.4	120.8	218.12		35.3			102.5	66.4	21.6	12.0							3.07													234		
53	Kongmun et al. 2009	15			47.9		35.2	103.4	186.36		27.2			96.6	66.1	21.4	12.5							3.09														83	
54	Ding et al. 2012	16	0.84					17.8	32.13	21.2	24.9															6.3													
55	Ding et al. 2012	16	0.72					6.9	12.5	10.3	9.6															4.8													
56	Ding et al. 2012	16	0.67					8.4	15.2	11.7	11.8															4.3													
57	Machmuller 2000	17	0.943	23		70.8		13.9	25.2	14.8	26.7			35.5	103.2	68.7	17.5	10.9	0.36	1.38	1.05	4.20			8.38														
58	Machmuller 2000	17	0.902	23		70		10.3	18.6	11.4	20.6			26.5	87.1	68.3	21.8	7.7	0.22	1.39	0.64	3.21			3.8														
59	Machmuller et al. 1999	18	1.178	23	65.8	71.3	74.3	57.8		41.2	35.0			57.8												8.15													
60	Machmuller et al. 1999	18	1.085	23	59.9	64.8	72.8	49.4		29.6	27.3			45.7												1.01													
61	Machmuller et al. 1999	18	0.889	23	69.4	72.8	79.7	49		11.1	12.5			15.2												0.3													

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# Methods



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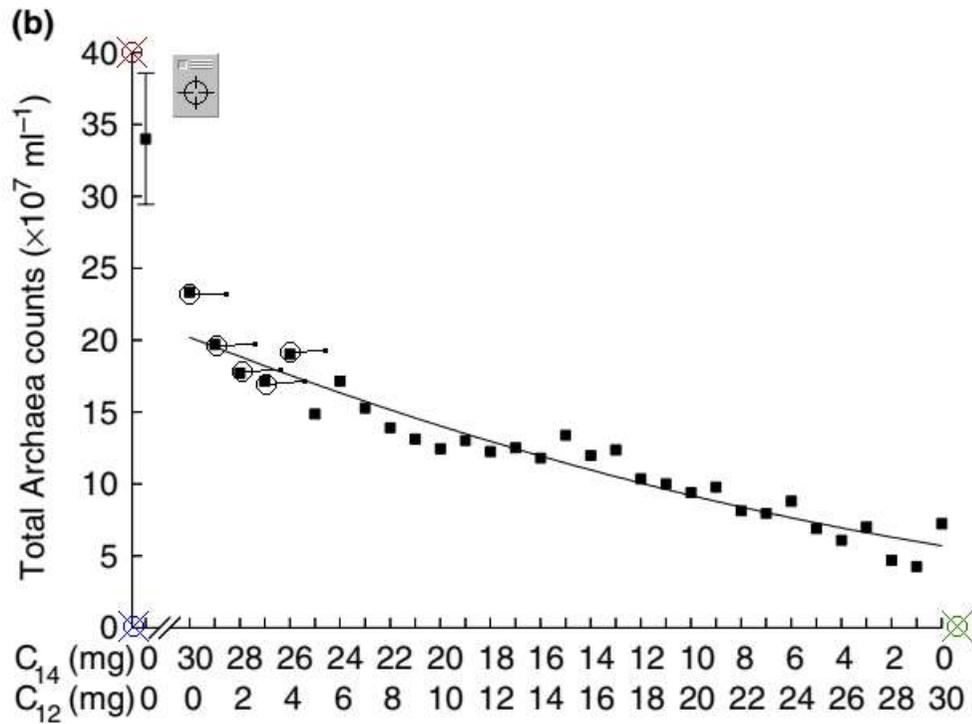
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# Methods



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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	Božić 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 production 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<sub>12:0</sub> and C<sub>14:0</sub>); 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 Anghthong (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<sub>12:0</sub> and C<sub>14:0</sub>); 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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SAS Studio interface showing a code editor with the following SAS code:

```
/** Import an XLSX file. */
PROC IMPORT DATAFILE="/folders/myfolders/mcfasept2019revised/SAS In vivo data.xlsx"
  OUT=WORK.InvivoMCFa
  DBMS=XLSX
  REPLACE;
RUN;

/** Print the results. */
PROC PRINT DATA=WORK.InvivoMCFa; RUN;

Title1 'The models Equation for MCFa invivo';

/* quadratic CH4mL*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4mL=MCFa|MCFa /solution;
random study;
title1 'quadratic CH4mL';
run;

/* linear CH4mL*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4mL=MCFa /solution;
random study;
title1 'linear CH4mL';
run;

/* Fit intercept-only linear design CH4mL*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4mL = ;
title1 'Fit Intercept-only Model CH4mL';
run;

/* quadratic CH4_DMI*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4_DMI=MCFa|MCFa /solution;
random study;
title1 'quadratic CH4_DMI';
run;

/* linear CH4_DMI*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4_DMI=MCFa /solution;
random study;
title1 'linear CH4_DMI';
run;

/* Fit intercept-only linear design CH4_DMI*/
proc mixed data=InvivoMCFa cl covtest method=type3;
class study;
model CH4_DMI = ;
title1 'Fit Intercept-only Model CH4_DMI';
run;
```

The interface also shows a file explorer on the left with folders like '2020 work', 'Paulownia', and 'mcfasept2019revised'. The bottom status bar shows 'Messages', 'User: saademo', and 'UTF-8'.

Windows taskbar with search bar 'Type here to search' and various application icons (Edge, Chrome, File Explorer, Mail, Word, Excel, PowerPoint, etc.). System tray shows date '5/11/2020' and time '4:59 PM'.



# Methods



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*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)				

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*The Mixed Procedure*

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr > Z	Alpha	Lower	Upper
Study	.70175	.14633	2.20	0.0275	0.05	7784.15	132266
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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# Methods



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- My Folders
  - 2020 work
    - Adam Gosia Polynova
    - Coleus RUSITEC-In vivo
    - Haihao
    - Hasan
    - Kometa Verko 2015
    - Lupine Magda
    - mcfasept2019revised
      - Archaea trial SAS.xlsx
      - ChangeCH4 SAS.xlsx
      - ChangeCH4revised2019analysis.sas
      - In vivo revised 2020.sas
      - MCFA In vitro ANOVA SAS.xlsx
      - MCFA In vitro script.sas
      - MCFA In vivo ANOVA SAS.xlsx
      - MCFA In vivo script.sas
      - MCFA Invitro revised2019.sas
      - MCFA InvitroANOVA revised2019.sas
      - SAS In vitro data.xlsx
      - SAS In vivo data.xlsx
      - Scatter Plot invitro.ctl
      - Scatter Plot invivo.ctl
  - Paulownia
    - In sacco paulownia
      - Paulownia insacco.sas
      - SAS Insacco results.xlsx
      - DAPI Paulownia in vitro.sas
      - Data Paulownia Invotr SAS.xlsx
      - Paulownia DAPI In vitro.xlsx
      - Paulownia in vitro.sas
      - SAS Dawid archaea.xlsx
    - Prof Adam
    - Prof Gosia dog
      - sasuser.v94
    - Slovakia analysis
    - test

- Tasks and Utilities
- Snippets
- Libraries
- File Shortcuts

CODE LOG RESULTS

```
1
2 /** Import an XLSX file. */
3
4 PROC IMPORT DATAFILE="/folders/myfolders/mcfasept2019revised/MCFA In vitro ANOVA SAS.xlsx"
5 OUT=WORK.MCFAInvitroANOVA
6 DBMS=XLSX
7 REPLACE;
8 RUN;
9
10 /** Print the results. */
11
12 PROC PRINT DATA=WORK.MCFAInvitroANOVA; RUN;
13
14
15 Title 'ANOVA of MCFA Invitro 2019';
16
17 /* ANOVA QMD*/
18 proc mixed data=MCFAInvitro2019 cl covtest method=type3;
19 class study Source;
20 model QMD=Source;
21 random study;
22 lsmeans Source/pdiff cl adjust=Tukey;
23 title1 'ANOVA QMD';
24 run;
25
26 /* ANOVA CPD*/
27 proc mixed data=MCFAINVITRO2019 cl covtest method=type3;
28 class study Source;
29 model CPD=Source;
30 random study;
31 lsmeans Source/pdiff cl adjust=Tukey;
32 title1 'ANOVA CPD';
33 run;
34
35 /* ANOVA NDFD*/
36 proc mixed data=MCFAINVITRO2019 cl covtest method=type3;
37 class study Source;
38 model NDFD=Source;
39 random study;
40 lsmeans Source/pdiff cl adjust=Tukey;
41 title1 'ANOVA NDFD';
42 run;
43
44 /* ANOVA pH*/
45 proc mixed data=MCFAINVITRO2019 cl covtest method=type3;
46 class study Source;
47 model pH=Source;
48 random study;
49 lsmeans Source/pdiff cl adjust=Tukey;
50 title1 'ANOVA pH';
51 run;
52
53 /* ANOVA TCOND*/
```

folders/myfolders/mcfasept2019revised/MCFA InvitroANOVA revised2019.sas UTF-8

Messages User: saademo 5:00 PM 5/11/2020



# Methods



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Browser tabs: Outlook, Google Scholar, SAS Studio

Browser address bar: localhost:10080/SASStudio/38/main?locale=en\_US&zone=GMT%252B02%253A00&http%3A%2F%2Flocalhost%3A10080%2FSASStudio%2F38%2Findex=

SAS Studio interface:

- Server Files and Folders: My folders, 2020 work, Adam Gośia Polynova, Coleus RUSITEC-In vivo, Haihao, Hassan, Kometa Verko 2015, Lupine Magda, mcfasept2019revised, Paulownia, Prof Adam, Prof Gośia dog, Slovakia analysis, test.
- Code Editor: 

```
1 /** Import an XLSX file. */
2
3 PROC IMPORT DATAFILE="/folders/myfolders/mcfasept2019revised/ChangeCH4 SAS.xlsx"
4           OUT=WORK.ChangeCH4
5           DBMS=XLSX
6           REPLACE;
7
8 RUN;
9
10 /** Print the results. */
11 PROC PRINT DATA=WORK.ChangeCH4 ; RUN;
12
13 /* quadratic change_of_CH4_OMD*/
14 proc mixed data=ChangeCH4 cl covtest method=type3;
15 class method;
16 model Change_CH4_OMD=MCFA|MCFA/solution outpm=quadraticChangeCH4;
17 random method;
18 title 'quadratic CH4_OMD';
19 run;
20
21 /* linear change_of_CH4_OMD*/
22 proc mixed data=ChangeCH4 cl covtest method=type3;
23 class method;
24 model Change_CH4_OMD=MCFA/solution;
25 random method;
26 title 'linear CH4_OMD';
27 run;
28
29 /* Fit intercept-only linear design DMD*/
30 proc mixed data=ChangeCH4 cl covtest method=type3;
31 class method;
32 model Change_CH4_OMD = ;
33 title 'Fit Intercept-only Model CH4_OMD';
34 run;
35
36 ods graphics / reset width=6in height=4in attrpriority=none imagemap ;
37 proc sgplot data=ChangeCH4 nouatlegend ;
38   reg x=MCFA y=in_vitro/ nomarkers lineattrs=(pattern=dot color=black thickness=8);
39   reg x=MCFA y=in_vivo/ nomarkers lineattrs=(pattern=mediumdashshortdash color=black thickness=8) degree=2;
40   reg x=MCFA y=Change_CH4_OMD/ nomarkers lineattrs=(pattern=solid color=black) degree=2;
41   styleattrs datasymbols=(triangle circle);
42   scatter x=MCFA y=Change_CH4_OMD / group=method markerattrs=(size=10);
43
44   xaxis min=-5 max=100 label="Dietary MCFA";
45   yaxis min=-100 max=100 label="CH4 Change/Digestible OM (%)";
46
47 run;
48 quit;
```

Taskbar: Windows Start button, search bar, taskbar icons (Edge, Chrome, File Explorer, Mail, Word, Excel, PowerPoint, SAS Studio, etc.), system tray (Messages, User: sasdemo, 4:58 PM, 5/11/2020).



# 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

Item	In vitro					In vivo				
	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 <sub>4</sub> (mL/d and L/d)	102	6.9	5.8	20.2	0.0	60	303	272	835	10.5
CH <sub>4</sub> /DMs (L/kg)	110	14.4	11.3	45.4	0.2	53	31.1	11.0	54.5	9.6
CH <sub>4</sub> /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 <sub>2</sub> (mol/100 mol VFA)	108	56.9	6.0	70.2	40.2	36	65.8	2.5	70.1	60.1
C <sub>3</sub> (mol/100 mol VFA)	109	22.6	6.8	39.5	11.4	40	21.2	3.0	27.1	13.8
C <sub>4</sub> (mol/100 mol VFA)	109	13.9	4.3	24.2	5.1	40	10.4	2.4	15.3	6.3
IsoC <sub>4</sub> (mol/100 mol VFA)	66	1.1	0.9	2.6	0.1	27	0.7	0.4	1.2	0.1
C <sub>5</sub> (mol/100 mol VFA)	88	4.0	2.7	9.6	0.6	27	1.7	0.5	2.7	1.0
IsoC <sub>5</sub> (mol/100 mol VFA)	69	2.8	1.5	5.8	0.5	27	1.3	0.5	2.1	0.4
C <sub>2</sub> :C <sub>3</sub>	105	2.7	1.1	5.3	0.1	38	3.2	0.5	4.2	2.3
H <sub>2</sub> (mmol/L)	56	0.9	1.1	4.0	0.0					
NH <sub>3</sub> (mmol/L)	70	12.6	6.7	25.6	2.1	23	8.3	3.5	18.0	2.5
Microorganism										
Protozoa (10 <sup>4</sup> /ml)	49	0.51	0.54	2.24	0.0	34	51.7	36.6	138.2	1.0
Bacteria (10 <sup>9</sup> /ml)	40	3.3	1.5	5.1	0.3	20	17.7	14.5	44.0	3.0
Archaea (10 <sup>7</sup> /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<sub>4</sub>: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<sub>2</sub>, acetate; C<sub>3</sub>, propionate; C<sub>4</sub>, butyrate; Iso-C<sub>4</sub>, isobutyrate; C<sub>5</sub>, valerate; Iso-C<sub>5</sub>:isovalerate; H<sub>2</sub>, 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

Response parameters	n	Model	Parameter estimates					RMSE	R <sup>2</sup>
			Intercept	SE Intercept	Slope	SE Slope	P value		
<b>In vitro digestibility (%)</b>									
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
<b>Rumen fermentation</b>									
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 <sub>4</sub> (mL)	102	L	9.51	1.6	-0.044	0.02	.03	3.55	0.62
CH <sub>4</sub> /DMs (L/kg)	110	L	18	2.3	-0.13	0.03	<.01	6	0.72
CH <sub>4</sub> /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 <sub>2</sub> (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 <sub>3</sub> (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 <sub>4</sub> (mol/100 mol VFA)	109	L	14.2	1	-0.015	0.008	.08	1.83	0.82
IsoC <sub>4</sub> (mol/100 mol VFA)	66	L	0.83	0.26	0.00002	0.0019	ns	0.32	0.86
C <sub>5</sub> (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 <sub>5</sub> (mol/100 mol VFA)	69	L	2.2	0.46	-0.0024	0.003	ns	0.48	0.89
C <sub>2</sub> :C <sub>3</sub>	105	Q	3.01	0.24	-0.021	0.007	<.01	0.6	0.7
					0.0002	0.0001	.03		
H <sub>2</sub> (mmol/L)	56	L	0.56	0.28	0.01	0.003	<.01	0.49	0.78
NH <sub>3</sub> (mmol/L)	70	Q	11.75	1.8	-0.11	0.05	.03	2.59	0.85
					0.0014	0.0008	.08		
<b>Microorganism</b>									
Protozoa (10 <sup>3</sup> /ml)	49	L	10.3	1.03	-0.19	0.03	<.01	3.16	0.65
Bacteria (10 <sup>7</sup> /ml)	40	L	3.27	0.49	-0.0009	0.006	ns	0.6	0.84
Archaea (10 <sup>7</sup> /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<sub>2</sub>, acetate; C<sub>3</sub>, propionate; C<sub>4</sub>, butyrate; C<sub>5</sub>, valerate; CH<sub>4</sub>, methane; CPD, digested crude protein; DMD, digested dry matter; DMs, dry matter substrate; H<sub>2</sub>, hydrogen; Iso-C<sub>4</sub>, isobutyrate; Iso-C<sub>5</sub>, isovalerate; Model, linear (L) or quadratic (Q); n, number of observations; NDFD, digested neutral detergent fibre; OMD, digested organic matter; R<sup>2</sup>, 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 <sup>a</sup>	50.3 <sup>b</sup>	–	54.2 <sup>b</sup>	47.5 <sup>b</sup>	–	–	–	<.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 <sup>a</sup>	18.1 <sup>b</sup>	21.8 <sup>ab</sup>	25.5 <sup>ab</sup>	15.0 <sup>b</sup>	27.9 <sup>ab</sup>	21.3 <sup>ab</sup>	15.3 <sup>b</sup>	<.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 <sub>4</sub> (mL)	102	9.75 <sup>a</sup>	10.2 <sup>a</sup>	–	9.21 <sup>abc</sup>	5.70 <sup>b</sup>	12.8 <sup>ab</sup>	4.77 <sup>c</sup>	–	<.01
CH <sub>4</sub> /DMs (L/kg)	110	18.5 <sup>a</sup>	12.3 <sup>b</sup>	15.8 <sup>ab</sup>	11.9 <sup>ab</sup>	10.9 <sup>b</sup>	18.6 <sup>ab</sup>	13.4 <sup>ab</sup>	13.5 <sup>ab</sup>	<.01
CH <sub>4</sub> /OMD (L/kg)	38	23.0 <sup>a</sup>	15.8 <sup>b</sup>	21.6 <sup>ab</sup>	–	13.7 <sup>b</sup>	21.9 <sup>ab</sup>	13.3 <sup>b</sup>	15.6 <sup>b</sup>	<.01
Total VFA (mmol/L)	106	81.7	76.7	80.2	80.6	75.2	85.9	79.1	82.2	ns
C <sub>2</sub> (mol/100 mol VFA)	105	57.9	56.2	58.0	55.9	56.3	59.5	54.4	56.2	ns
C <sub>3</sub> (mol/100 mol VFA)	109	21.5 <sup>b</sup>	24.4 <sup>a</sup>	21.3 <sup>ab</sup>	24.6 <sup>ab</sup>	24.3 <sup>a</sup>	22.1 <sup>ab</sup>	22.5 <sup>ab</sup>	23.5 <sup>ab</sup>	.07
C <sub>4</sub> (mol/100 mol VFA)	108	14.1	14.0	14.9	14.2	13.2	13.1	15.0	14.5	ns
IsoC <sub>4</sub> (mol/100 mol VFA)	66	0.86	0.64	–	–	0.84	0.85	0.80	–	ns
C <sub>5</sub> (mol/100 mol VFA)	79	3.57 <sup>b</sup>	2.89 <sup>b</sup>	4.42 <sup>ab</sup>	–	3.21 <sup>b</sup>	3.66 <sup>b</sup>	5.84 <sup>a</sup>	3.85 <sup>b</sup>	.03
Iso-C <sub>5</sub> (%)	69	2.28	1.62	–	–	2.18	2.06	2.06	1.65	ns
C <sub>2</sub> :C <sub>3</sub>	105	3.04 <sup>a</sup>	2.74 <sup>ab</sup>	3.45 <sup>a</sup>	2.61 <sup>ab</sup>	2.33 <sup>b</sup>	2.89 <sup>ab</sup>	2.71 <sup>ab</sup>	2.99 <sup>ab</sup>	.03
H <sub>2</sub> (mmol/L)	50	0.53 <sup>c</sup>	0.77 <sup>abc</sup>	0.53 <sup>bc</sup>	–	1.37 <sup>ab</sup>	0.49 <sup>c</sup>	1.84 <sup>a</sup>	1.24 <sup>abc</sup>	.02
NH <sub>3</sub> (mmol/L)	70	11.7	9.32	10.6	–	10.0	13.2	9.23	9.52	ns
Microorganism										
Protozoa (10 <sup>3</sup> /ml)	42	10.3 <sup>a</sup>	2.59 <sup>b</sup>	2.21 <sup>b</sup>	–	1.52 <sup>b</sup>	9.10 <sup>a</sup>	3.26 <sup>b</sup>	–	<.01
Bacteria (10 <sup>9</sup> /ml)	40	3.30	2.98	3.45	–	3.39	3.55	3.30	4.00	ns
Archaea (10 <sup>7</sup> /ml)	50	15.1 <sup>a</sup>	6.80 <sup>ab</sup>	9.75 <sup>ab</sup>	–	1.48 <sup>b</sup>	9.10 <sup>a</sup>	0.00 <sup>b</sup>	9.74 <sup>ab</sup>	<.01

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

Abbreviations: C<sub>2</sub>, acetate; C<sub>3</sub>, propionate; C<sub>4</sub>, butyrate; C<sub>5</sub>, valerate; CanO, canola oil enriched with lauric acid; CH<sub>4</sub>, methane; CO, coconut oil; CPD, digested crude protein; DMD, digested dry matter; DMs, dry matter substrate; H<sub>2</sub>, hydrogen; Iso-C<sub>4</sub>, isobutyrate; Iso-C<sub>5</sub>, 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

Response Parameters	n	Model	Parameter estimates					RMSE	R <sup>2</sup>
			Intercept	SE Intercept	Slope	SE Slope	p value		
DMI (kg/d)	65	Q	10.2	1.9	-0.072	0.024	<.01	1.05	0.98
					0.0008	0.0004	.05		
<b>In vivo digestibility (%)</b>									
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
<b>Rumen fermentation</b>									
pH	32	L	6.61	0.1	-0.002	0.001	.08	0.12	0.83
CH <sub>4</sub> (L/d)	60	L	303	66.6	-1.58	0.5	<.01	67.6	0.94
CH <sub>4</sub> /DMI (L/kg)	53	Q	33.8	2.79	-0.32	0.1	<.01	3.89	0.87
					0.0029	0.0014	.05		
CH <sub>4</sub> /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 <sub>2</sub> (mol/100 mol VFA)	36	L	65.6	0.7	-0.011	0.013	ns	1.28	0.73
C <sub>3</sub> (mol/100 mol VFA)	40	L	20.5	0.8	0.03	0.02	.06	1.65	0.7
C <sub>4</sub> (mol/100 mol VFA)	40	L	10.8	0.7	-0.01	0.01	ns	1.02	0.81
IsoC <sub>4</sub> (mol/100 mol VFA)	27	L	0.78	0.13	-0.003	0.001	.03	0.1	0.91
					0.0002	0.0001	.02		
C <sub>5</sub> (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 <sub>5</sub> (mol/100 mol VFA)	27	L	1.46	0.19	-0.006	0.002	<.01	0.18	0.87
C <sub>2</sub> :C <sub>3</sub>	38	L	3.27	0.12	-0.006	0.003	.05	0.31	0.52
NH <sub>3</sub> (mmol/L)	23	L	10.5	1.3	-0.08	0.02	<.01	0.91	0.93
<b>Microorganism</b>									
Protozoa (10 <sup>3</sup> /ml)	34	L	7.14	0.99	-0.09	0.02	<.01	1.87	0.73
Bacteria (10 <sup>7</sup> /ml)	20	L	18	7.2	-0.05	0.04	ns	2.77	0.96
Archaea (10 <sup>7</sup> /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<sup>2</sup>, R-square; DMI, dry matter intake; DMD, digested dry matter; OMD, digested organic matter; CPD, digested crude protein; NDFD, digested neutral detergent fibre; CH<sub>4</sub>, methane; VFA, volatile fatty acids; C<sub>2</sub>, acetate; C<sub>3</sub>, propionate; C<sub>4</sub>, butyrate; Iso-C<sub>4</sub>, isobutyrate; C<sub>5</sub>, valerate; Iso-C<sub>5</sub>, 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 <sup>a</sup>	8.90 <sup>b</sup>	8.75 <sup>b</sup>	10.2 <sup>ab</sup>	10.0 <sup>ab</sup>	.01
In vivo digestibility (%)							
DMD	30	67.8 <sup>a</sup>	64.8 <sup>b</sup>	69.3 <sup>a</sup>	—	—	.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 <sup>a</sup>	6.64 <sup>a</sup>	6.56 <sup>a</sup>	6.15 <sup>b</sup>	—	<.01
CH <sub>4</sub> (L/d)	60	320 <sup>a</sup>	231 <sup>b</sup>	285 <sup>ab</sup>	296 <sup>ab</sup>	284 <sup>ab</sup>	.04
CH <sub>4</sub> /DMI (L/kg)	53	34.6 <sup>a</sup>	27.4 <sup>b</sup>	33.7 <sup>ab</sup>	21.3 <sup>bc</sup>	27.0 <sup>abc</sup>	<.01
CH <sub>4</sub> /OMD (L/kg)	36	42.9 <sup>a</sup>	33.0 <sup>b</sup>	41.5 <sup>ab</sup>	30.2 <sup>ab</sup>	—	<.01
Total VFA (mmol/L)	34	112 <sup>a</sup>	99 <sup>b</sup>	110 <sup>a</sup>	84 <sup>c</sup>	111 <sup>ab</sup>	<.01
C <sub>2</sub> (mol/100 mol VFA)	37	65.8	65.2	64.4	65.6	62.3	ns
C <sub>3</sub> (mol/100 mol VFA)	40	20.1 <sup>b</sup>	21.0 <sup>ab</sup>	22.3 <sup>ab</sup>	24.6 <sup>a</sup>	23.2 <sup>ab</sup>	.02
C <sub>4</sub> (mol/100 mol VFA)	40	10.8 <sup>a</sup>	11.1 <sup>a</sup>	10.6 <sup>a</sup>	7.5 <sup>b</sup>	10.1 <sup>ab</sup>	.02
IsoC <sub>4</sub> (mol/100 mol VFA)	27	0.77 <sup>a</sup>	0.79 <sup>a</sup>	0.71 <sup>a</sup>	0.40 <sup>b</sup>	0.70 <sup>ab</sup>	.01
C <sub>5</sub> (mol/100 mol VFA)	24	1.68 <sup>bc</sup>	1.88 <sup>a</sup>	1.68 <sup>b</sup>	1.49 <sup>c</sup>	1.88 <sup>ab</sup>	.01
Iso-C <sub>5</sub> (mol/100 mol VFA)	27	1.47	1.23	1.34	1.06	1.68	ns
C <sub>2</sub> :C <sub>3</sub>	37	3.38 <sup>a</sup>	3.07 <sup>b</sup>	2.93 <sup>bc</sup>	2.51 <sup>c</sup>	2.74 <sup>bc</sup>	<.01
NH <sub>3</sub> (mM)	23	9.61 <sup>b</sup>	7.25 <sup>c</sup>	7.33 <sup>bc</sup>	5.98 <sup>c</sup>	18.0 <sup>a</sup>	<.01
Microorganism							
Protozoa (10 <sup>5</sup> /ml)	34	8.46 <sup>a</sup>	4.24 <sup>b</sup>	1.25 <sup>b</sup>	4.78 <sup>b</sup>	6.33 <sup>ab</sup>	<.01
Bacteria (10 <sup>9</sup> /ml)	20	18.1	16.5	—	16.7	—	ns
Archaea (10 <sup>7</sup> /ml)	16	97.4	59.3	—	84.0	—	ns

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

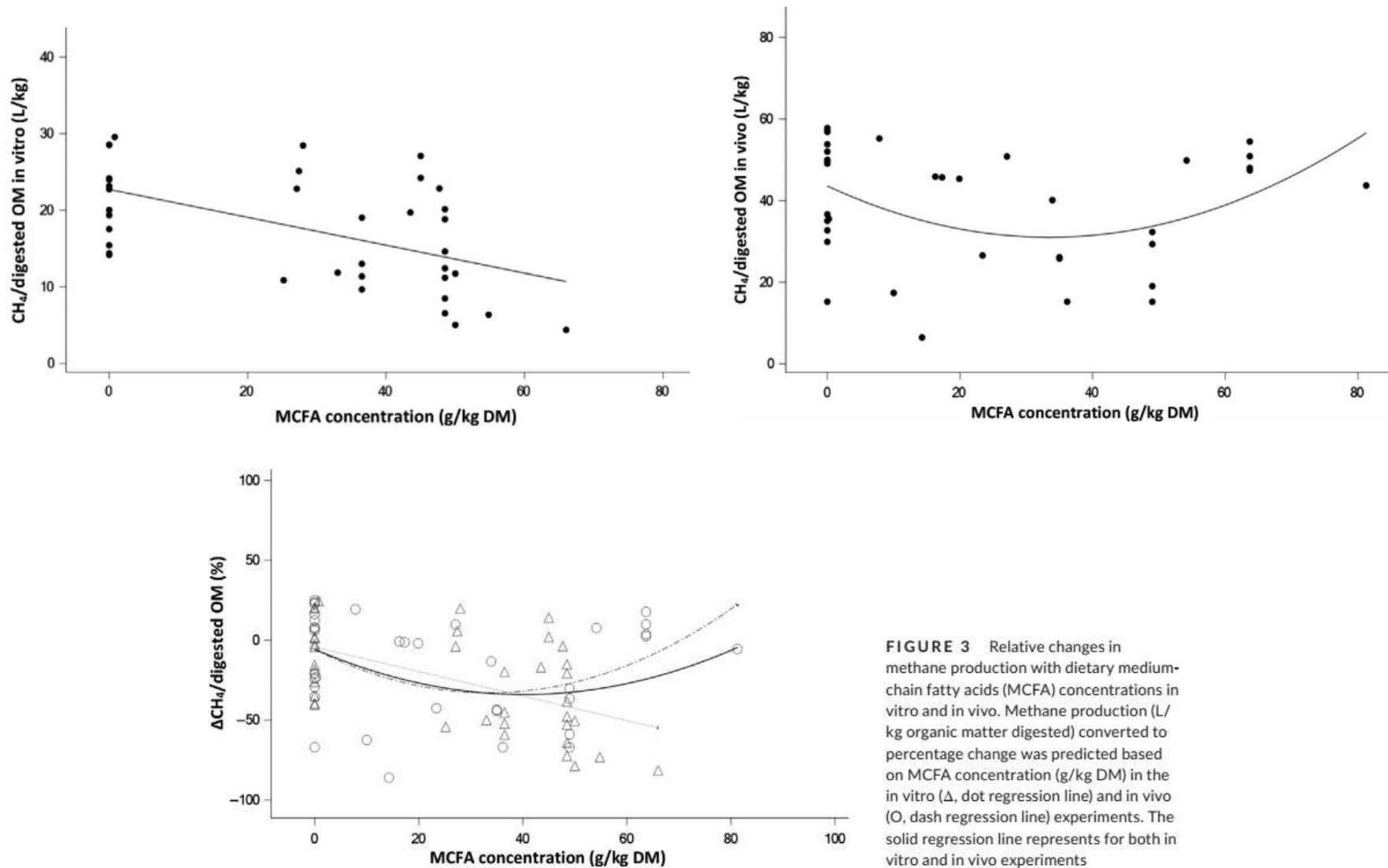
n, number of observations; CO, coconut oil; LA, lauric acid; MA, myristic acid; LMA, mixed lauric and myristic acids; DMI, dry matter intake; DMD, digested dry matter; OMD, digested organic matter; CPD, digested crude protein; NDFD, digested neutral detergent fibre; CH<sub>4</sub>, methane; VFA, volatile fatty acids; C<sub>2</sub>, acetate; C<sub>3</sub>, propionate; C<sub>4</sub>, butyrate; Iso-C<sub>4</sub>, isobutyrate; C<sub>5</sub>, valerate; Iso-C<sub>5</sub>, isovalerate.



# Results



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# Conclusion





- Increasing the MCFA concentrations caused a decrease in CH<sub>4</sub> 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<sub>4</sub> 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.



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**Example of  
other project**



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**Thank you  
for  
Your attentions!**



**Yuk kerja bareng!**