SEAMEO SEARCA

PUBLIC LECTURE

"Natural Feed Additive for Enhancing Production Performance, Mitigating Methane Emission and Improving Product Quality of Livestock"

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Bogor, 14 February 2018

SEARCA Regional Professorial Chair

The Southeast Asian Regional Center for Graduate Study and Research in Agriculture

SEARCA:
The Southeast Asian Regional Center for Graduate Study and Research in Agriculture

About

Established in 1966 by the Southeast Asian Ministers of Education Organization (SEAMEO)

The SEAMEO center of excellence in agriculture

Mandate:

To provide to the participating countries high quality graduate study in agriculture; promote, undertake, and coordinate research programs related to the needs and problems of the Southeast Asian region; and disseminate the findings of agricultural research and experimentation

Opportunity

- Full graduate (master and PhD) scholarship
- PhD research scholarship
- University Consortium grant
- Faculty and student mobility grant
- Seed fund for research and training
- Research fellowship program
- Dioscoro L. Umali Achievement Award in Agricultural Development
- **SEARCA Regional Professorial Chair Award**
- Others (http://www.searca.org)

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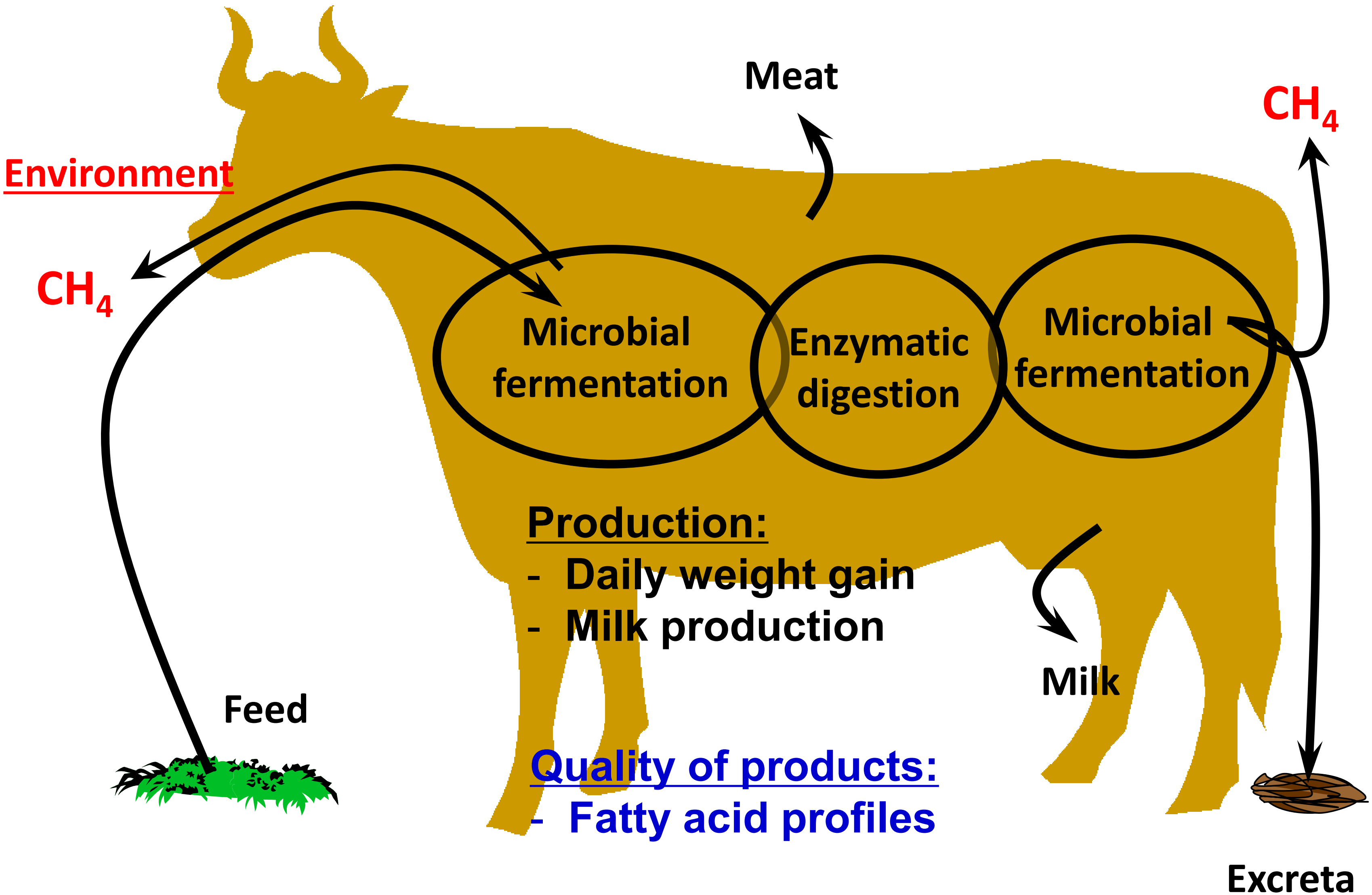
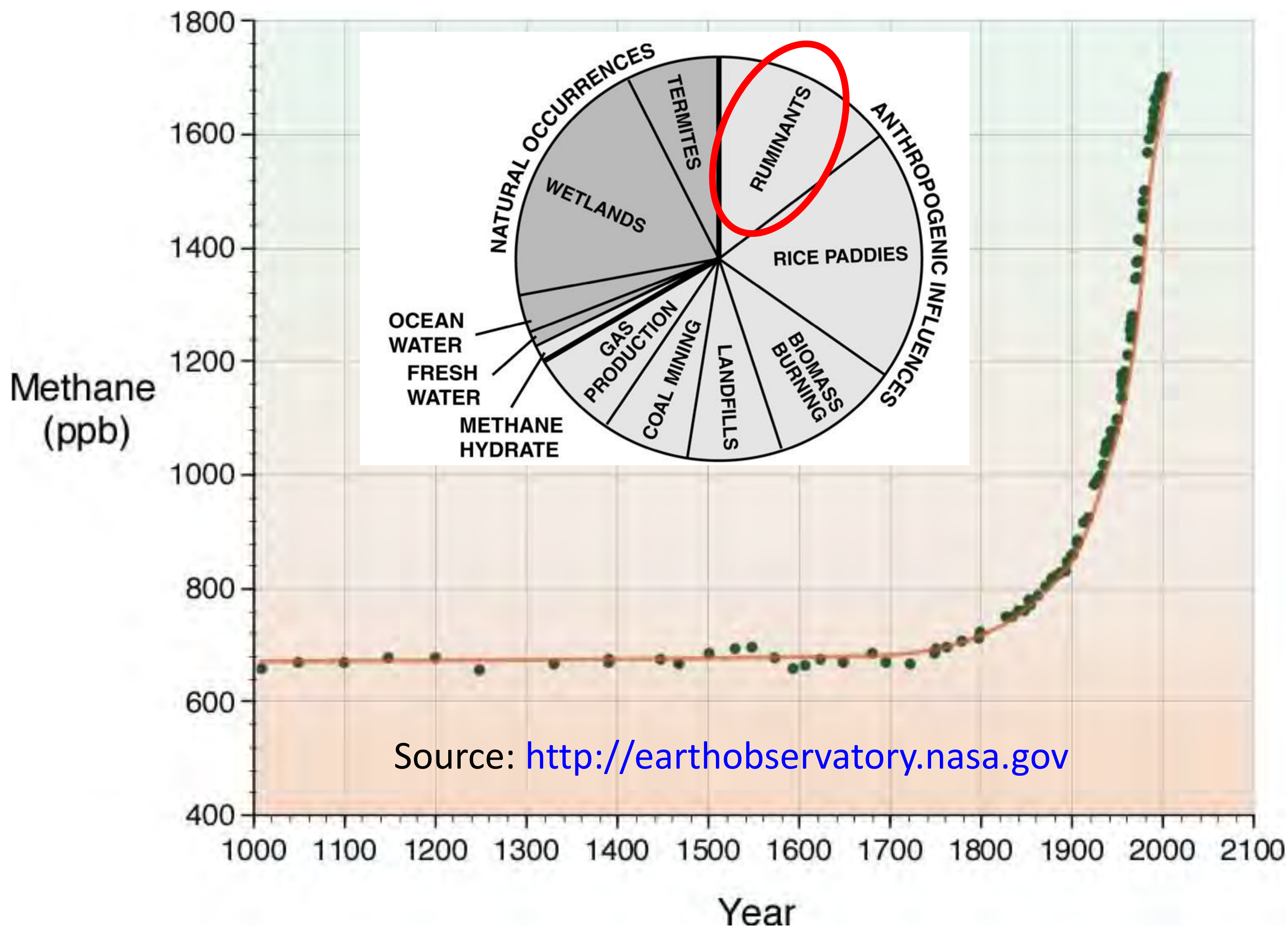
E. Effects on product quality
   - Evidences
   - Mode of action
F. An on going study

A. Introduction


Meat
CH_4
Environment
CH_4
Microbial fermentation
Enzymatic digestion
Microbial fermentation
Production:
- Daily weight gain
- Milk production
Milk
Feed
Quality of products:
- Fatty acid profiles
Excreta

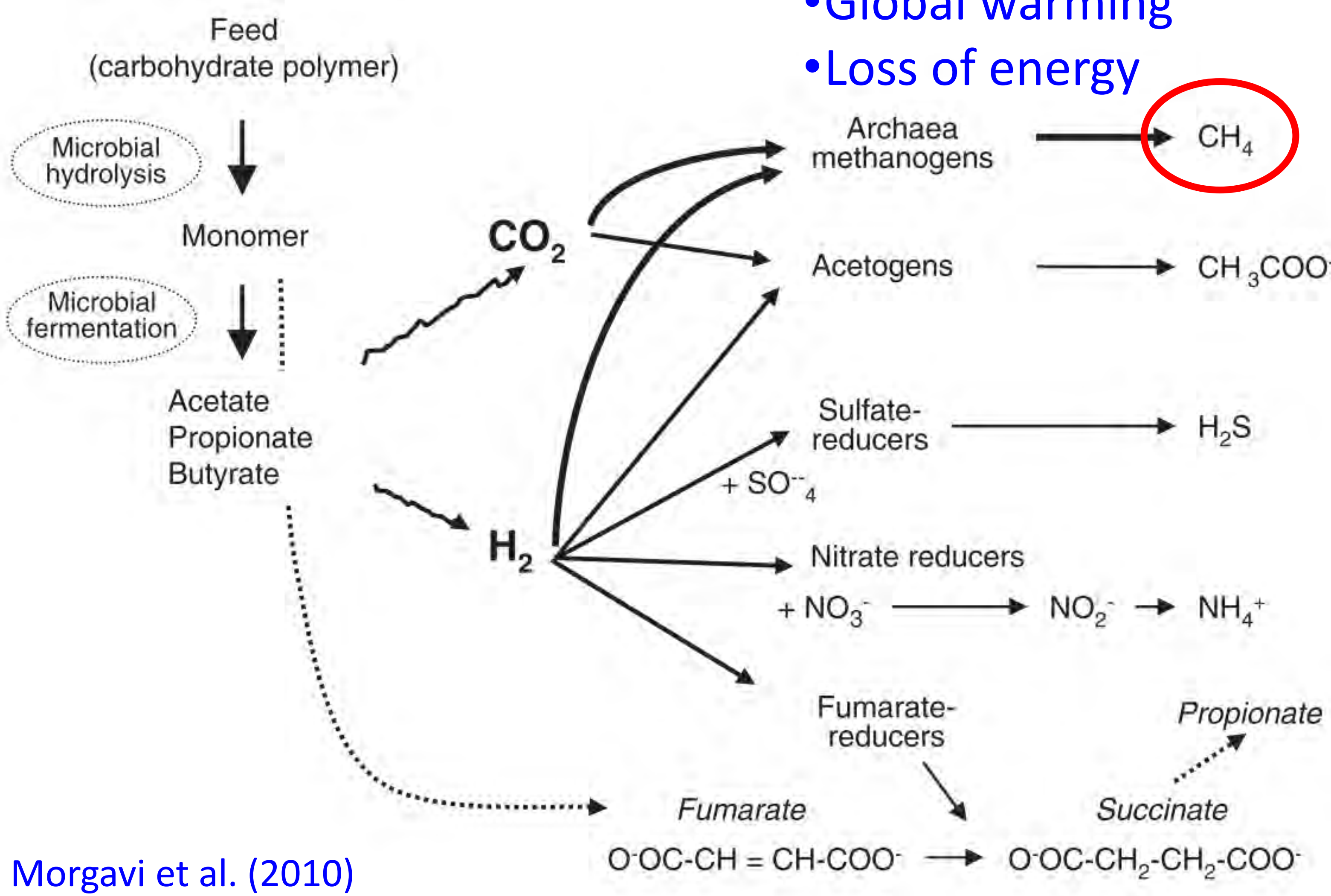
GHG emission



Source: http://earthobservatory.nasa.gov

Methane formation

- Global warming
- Loss of energy



Source: Morgavi et al. (2010)
Animal 4, 1024-1036

Product quality

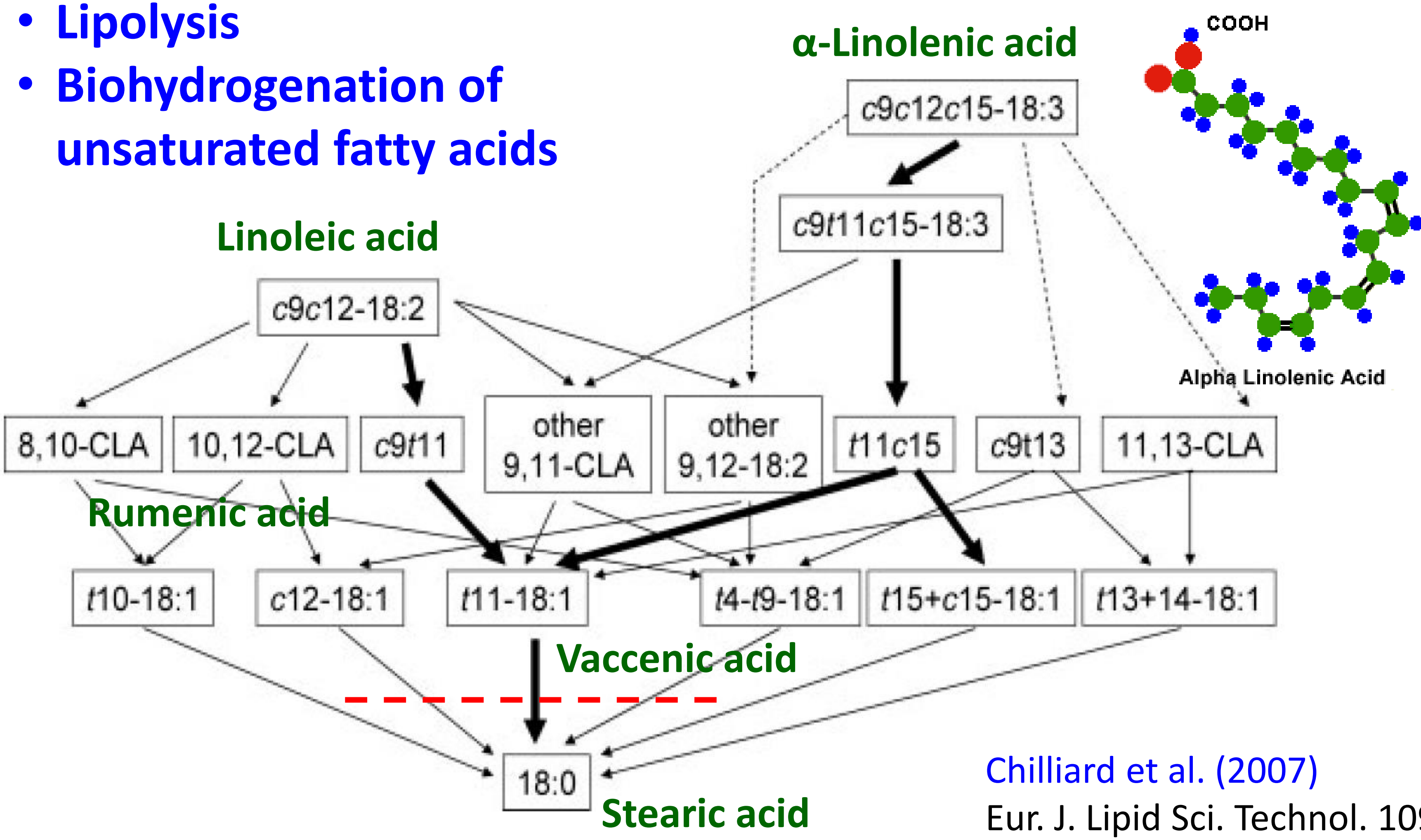
de Almeida et al. (2006)
Braz. J. Pharm. Sci., 109-117

TABLE II - Fatty Acid Composition (mg/100 g) of Raw Chicken and Beef [a]

Composition	Beef cuts		Dark chicken meat[a]	Anova P[b]
	Semimembranosus	*Biceps femoris*		
Myristic acid (14:0)	99 ± 9	356 ± 11	29 ± 3	<0.001[d]
Palmitic acid (16:0)	958 ± 61	2804 ± 198	1097 ± 124	<0.001[e]
Stearic acid (18:0)	498 ± 46	1450 ± 119	302 ± 22	<0.001[d]
Total saturated fatty acids	1555 ± 116	4610 ± 328	1428 ± 124	<0.001[e]
Palmitoleic acid (16:1n-7)	207 ± 173	639 ± 87	298 ± 56	<0.010[e]
Oleic acid (18:1n-9)	1108 ± 118	3010 ± 80	1366 ± 77	<0.001[d]
n-9 monounsaturated	1108 ± 118	3010 ± 80	1366 ± 77	<0.001[d]
Total monounsaturated fatty acid	1315 ± 173	3649 ± 87	1664 ± 77	<0.001[d]
Linoleic acid (18:2n-6)	35 ± 24	183 ± 24	728 ± 94	<0.001[f]
Gamma-linolenic (18:3n-6)	1	39 ± 10	1	<0.001[e]
α-Linolenic acid (18:3n-3)	32 ± 10	22 ± 5	25 ± 4	0.263
Arachidonic acid (20:4n-6)	21 ± 5	15 ± 1	46 ± 3	0.110
Eicosapentaenoic acid (EPA, 22:5n-3)	0.1	0.1	23 ± 3	0.001[f]
Docosahexaenoic acid(DHA, 22:6n-3)	0.1	0.1	14 ± 1	0.014[f]
n-3 polyunsaturated fatty acid	32 ± 10	22 ± 5	62 ± 4	0.001[f]
n-6 polyunsaturated fatty acid	57 ± 24	237 ± 24	775 ± 94	<0.001[f]
Total polyunsaturated fatty acid	89 ± 24	259 ± 24	837 ± 94	<0.001[f]

Lipid metabolism

- Lipolysis
- Biohydrogenation of unsaturated fatty acids



Chilliard et al. (2007)
Eur. J. Lipid Sci. Technol. 109, 828-855

1. Enhancing production performance
2. Mitigating methane emission
3. Improving product quality

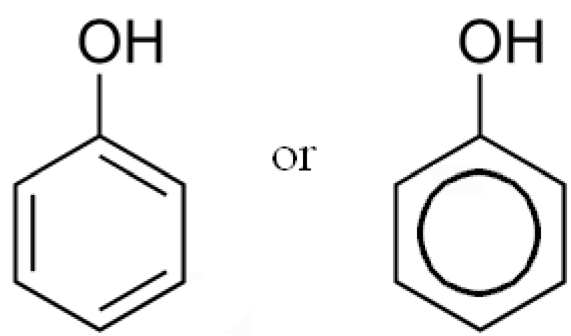
Natural feed additives:

1. Polyphenols
2. Saponins
3. Essential oils

B. Characteristics of polyphenols

Polyphenols

<u>Definition of phenolics:</u> a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group.



Basic structure of phenolics

Phenolics

Tannin phenolics
(which bind proteins)

Nontannin phenolics
(which do not bind proteins)

Hydrolysable Tannins
(Esters of polyhydroxy Alcohol for example sugar and Gallic or Ellagic acids)

Gallic acid or Ellagic acid

+

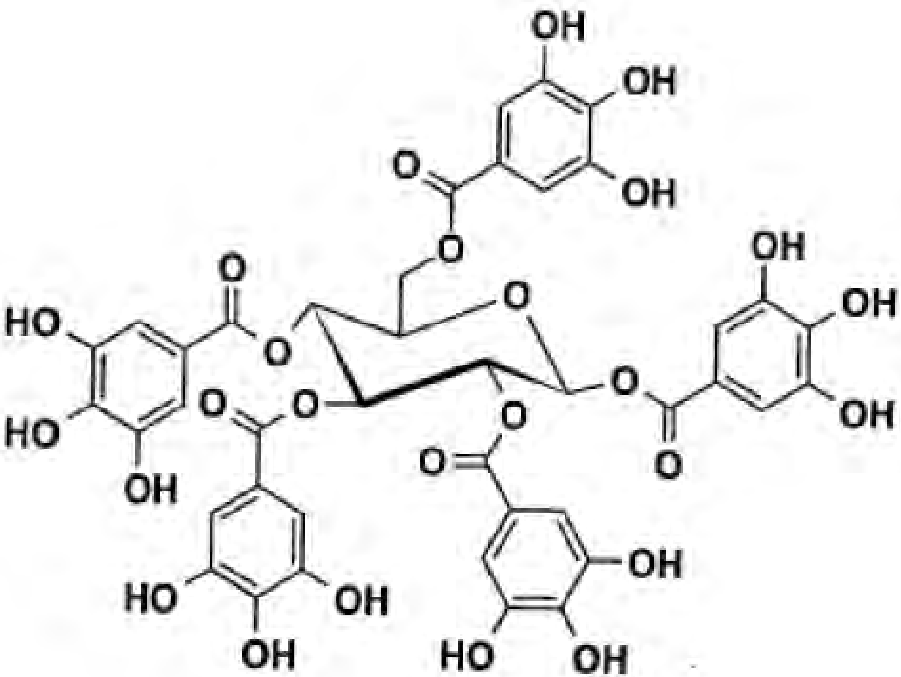
Sugar

Condensed Tannins
(Polymers of Flavan 3-ols and/or Flavan 3,4-diols)

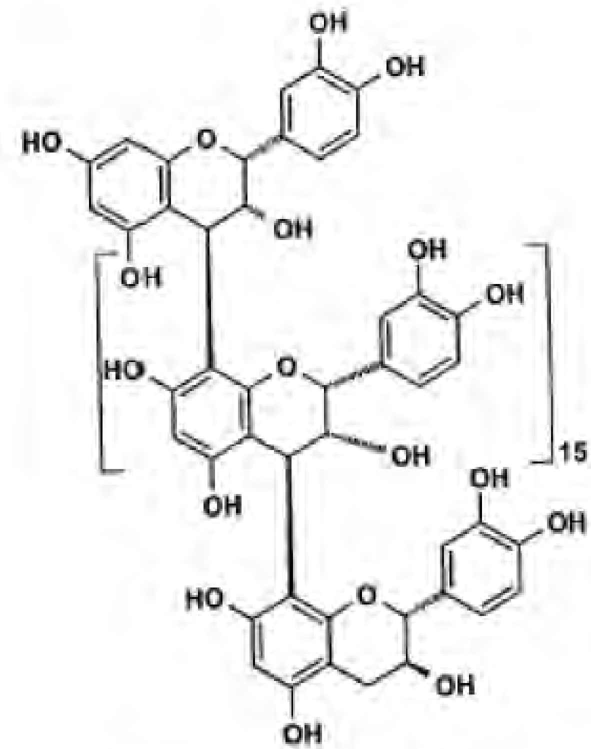
Difficult to break

Classification

Structure



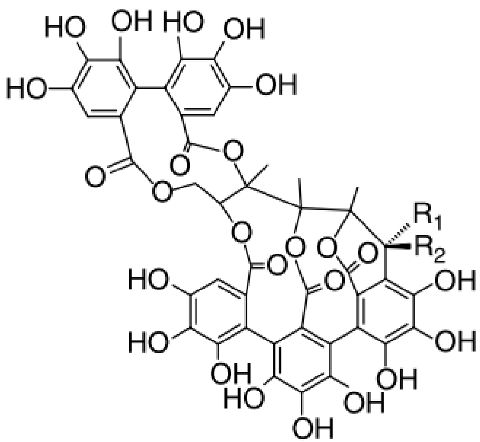
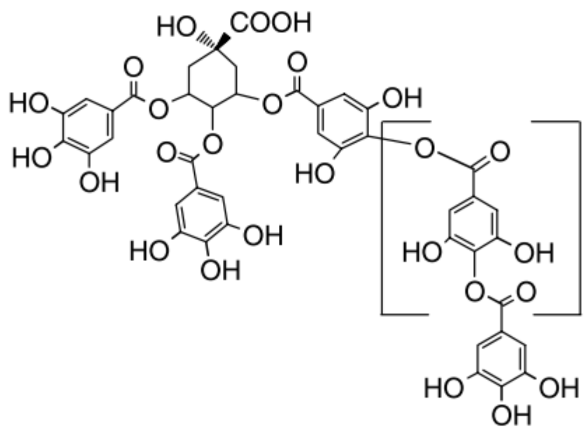
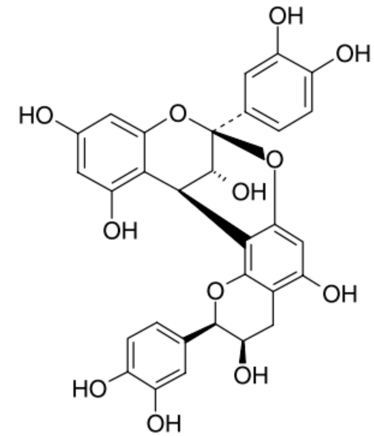
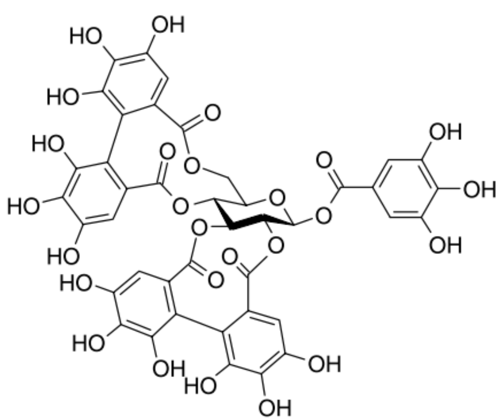
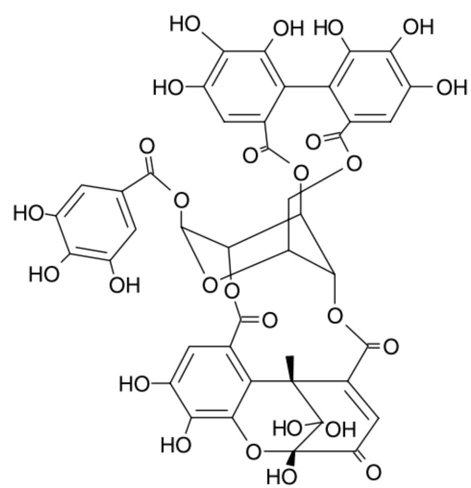
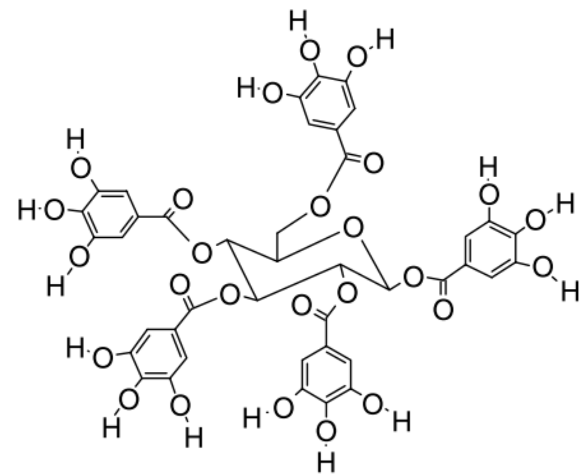
Hydrolysable tannin



Condensed tannin

Source: McSweeney et al. (2001)
Anim. Feed Sci. Technol. 91, 83-93

Diversity of tannin structures

Source: Mueller-Harvey (2006)

J. Sci. Food Agric. 86, 2010-2037

Synthesis

Why plants produce phenolics?

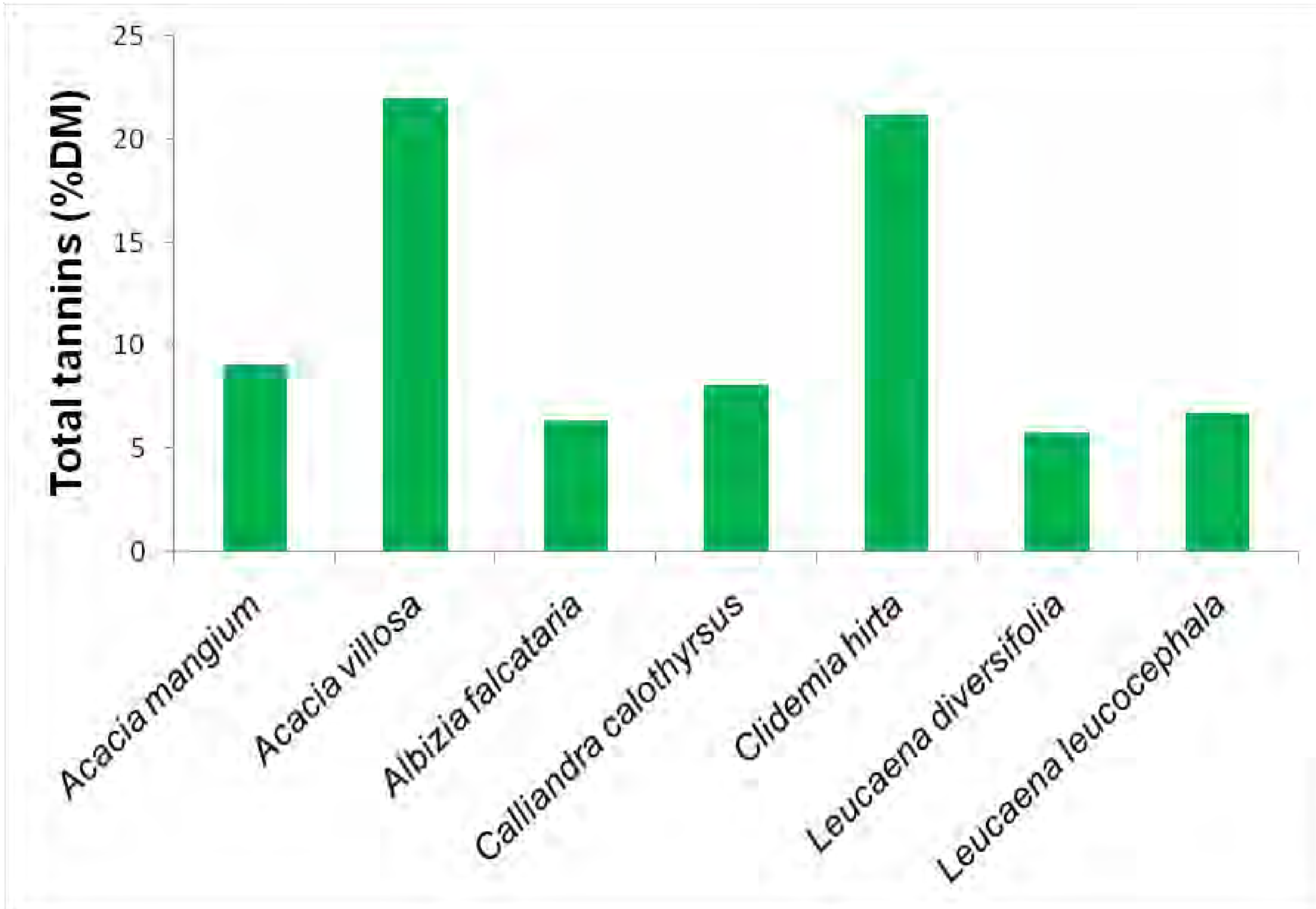
A strategy adopted by plants to deter attack by microorganisms, insects and higher animals.

Factors affecting tannin levels:

- Nutrient stress (N, P, K, S deficiencies)
- High light intensity
- High temperature
- Severe drought
- Tissue damage

Increase tannin levels

Source: Jayanegara et al. (2011)
Anim. Feed Sci. Technol. 163, 231-243



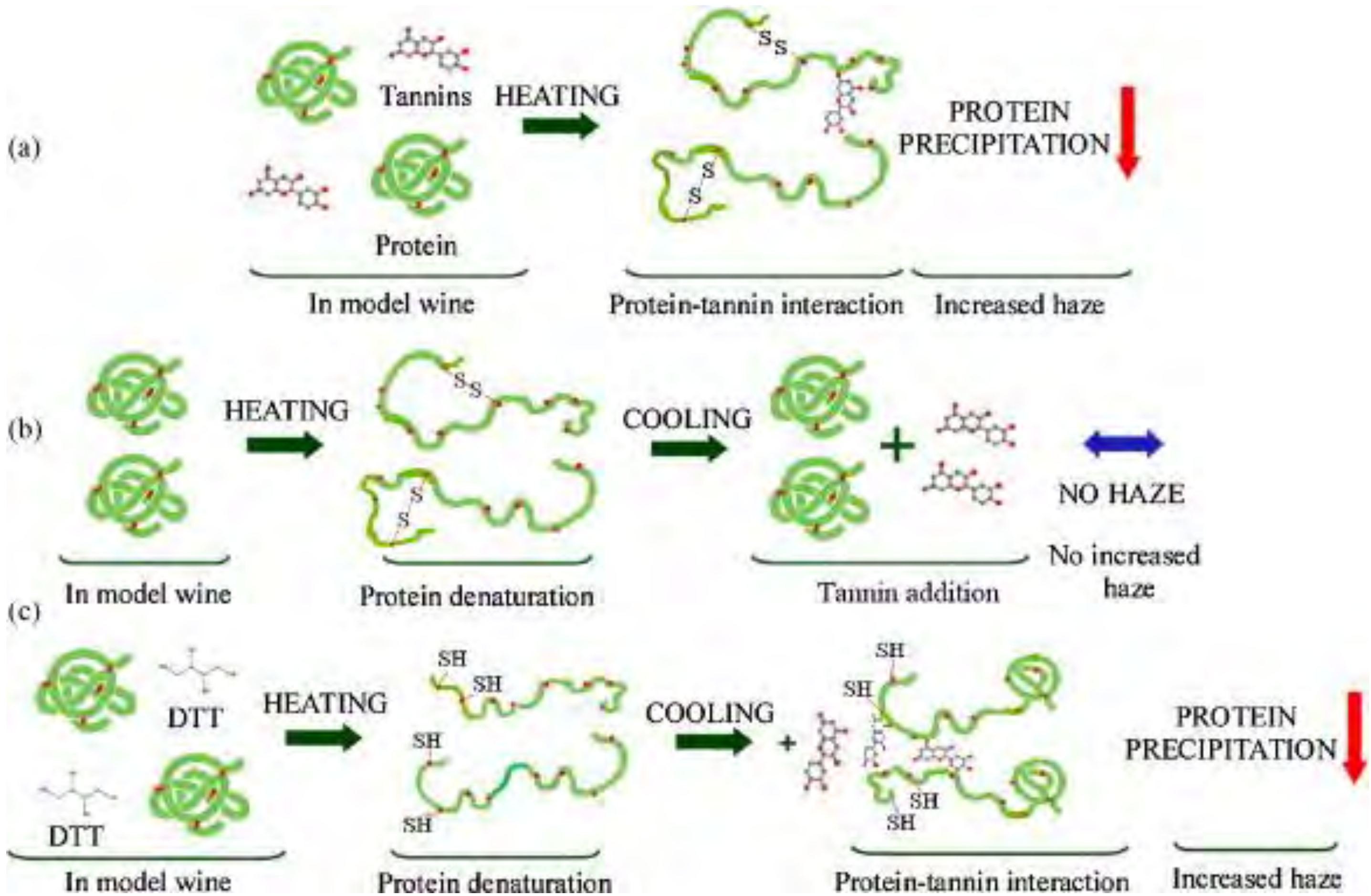
Interaction

Multiple hydroxyl groups: enable phenolics to form complexes with proteins, polysachharides and minerals.

Tannin-protein complex:

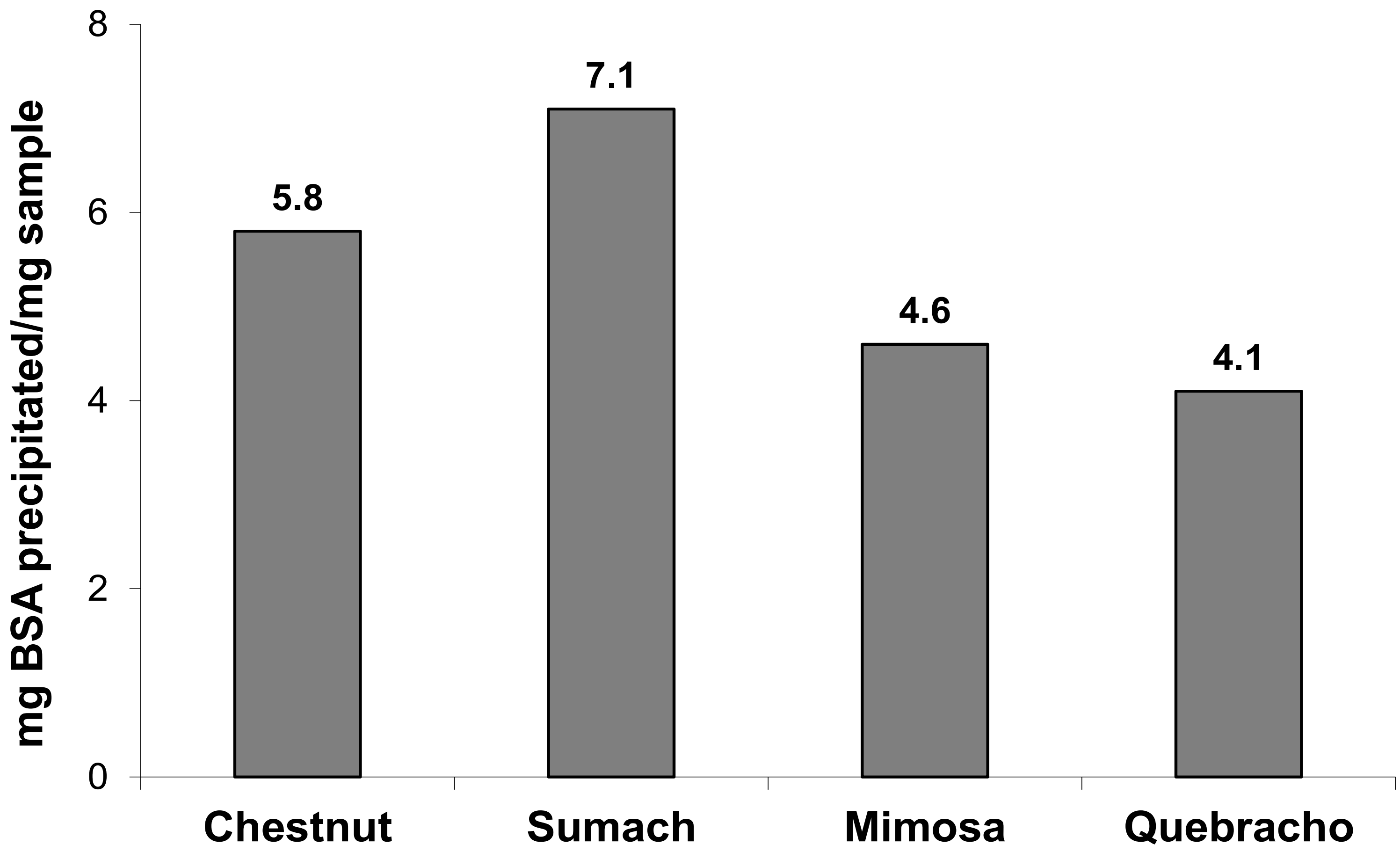
1. Hydrogen bonds: free phenolic hydroxyl groups
2. Hydrophobic bonds: aromatic ring structures
3. Covalent bonds: polymerization reactions due to heating, exposure to UV radiation and the action of polyphenol oxidase

Protein precipitation by tannins



Marangon et al. (2010)
Analytica Chimica Acta 660, 110-118

Jayanegara et al. (2015)
Animal Feed Science and Technology 209, 60-68



C. Effects on production performance

Table 3. Effect of dietary forage source on production

	Silage source[1]				
CT in diet (g/kg DM)	0	1.2	5.0	7.3	9.5
Item	AL	RC	LBFT	NBFT	HBFT
N intake, g/d	713	730	685	637	684
DMI, kg/d	24.7	25.6	24.7	24.0	25.2
BW change, kg/d	−0.01	0.03	0.53	0.15	0.43
Milk, kg/d	30.9^{c}	30.8^{c}	33.5^{b}	34.6^{ab}	35.4^{a}
Milk/DMI	1.27^{bc}	1.24^{c}	1.38^{abc}	1.49^{a}	1.42^{ab}
ECM, kg/d	30.0^{bc}	29.3^{c}	31.8^{ab}	33.4^{a}	33.8^{a}
ECM/DMI	1.23^{bc}	1.18^{c}	1.30^{abc}	1.43^{a}	1.37^{ab}
Fat, %	3.74	3.72	3.57	3.86	3.74
Fat, kg/d	1.18^{bc}	1.16^{c}	1.23^{abc}	1.35^{a}	1.32^{ab}
True protein, %	3.19^{a}	3.06^{b}	3.19^{a}	3.14^{a}	3.21^{a}
Protein, kg/d	1.00^{c}	0.95^{c}	1.07^{b}	1.08^{ab}	1.14^{a}
Lactose, %	4.69	4.73	4.65	4.71	4.71
Lactose, kg/d	1.48^{b}	1.48^{b}	1.60^{a}	1.62^{a}	1.69^{a}
SNF, %	8.78	8.68	8.75	8.74	8.82
SNF, kg/d	2.77^{c}	2.71^{c}	2.98^{b}	3.01^{ab}	3.15^{a}
Milk-N/N-intake, %	22.3^{bc}	21.0^{c}	24.9^{ab}	27.5^{a}	26.6^{a}
MUN, mg/dL	11.4^{a}	11.0^{ab}	11.4^{a}	9.7^{c}	10.1^{bc}
Plasma urea-N, mg/dL	17.0^{a}	14.2^{b}	13.8^{b}	12.7^{b}	14.6^{b}

[a–c]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birds
birdsfoot trefoil.

Performance of dairy cows

Hymes-Fecht et al. (2013)
J. Dairy Sci. 96, 460-469

Table 2 *DMI, live weight change, milk production, MUN and faecal N concentration of cows grazing fresh pasture (CC wattle (Acacia mearnsii) CT extract at low (LCT), medium (MCT) and high (HCT) concentrations*

	CONT ($n = 20$)	LCT ($n = 6$)	MCT ($n = 6$)	HCT ($n = 6$)
CT, intake and live weight data				
CT in drench (g/day)	0	111	222	444
DMI (kg/day)[1]	16.7	17.4	15.8	15.5
CT dose rate (% of DMI)	–	0.6	1.4	2.9
Live weight change (kg/day)[2]	0.31	0.39	0.19	0.26
Production per cow (kg/day)				
Fluid milk yield	23.9[ab]	24.8[a]	23.0[bc]	21.9[c]
Milksolids (fat + protein)	1.80[ab]	1.87[a]	1.72[bc]	1.64[c]
Milk composition (%)				
Fat	4.06	4.07	3.99	4.02
Protein	3.48	3.49	3.53	3.50
Lactose	4.85	4.86	4.80	4.85
Milk urea and faecal nitrogen				
MUN (mmol/l)	6.02[a]	5.97[a]	5.49[b]	5.55[b]
Faecal nitrogen (% of DM)	3.68[b]	3.87[a]	3.82[a]	3.76[ab]

DMI = dry matter intake; MUN = milk urea nitrogen; CT = condensed tannins; DM = dry matter.
Data are means for the 3-week measurement period in the Drench experiment.
[1]DMI (excluding CT) estimated by back-calculation using SCA (1990); assuming a dietary ME content of 12.1 MJ/kg DM.
[2]Based on regression of weekly live weights over 4 weeks preceding and succeeding the experimental period.
[a,b]Values within a row with same superscripts are not significantly different at 5% significance levels.

Griffiths et al. (2013)
Animal 7, 1789-1795

Beef cattle

Table 2
Effects of source of tannin on animal performance and feed efficiency traits in finishing steers.

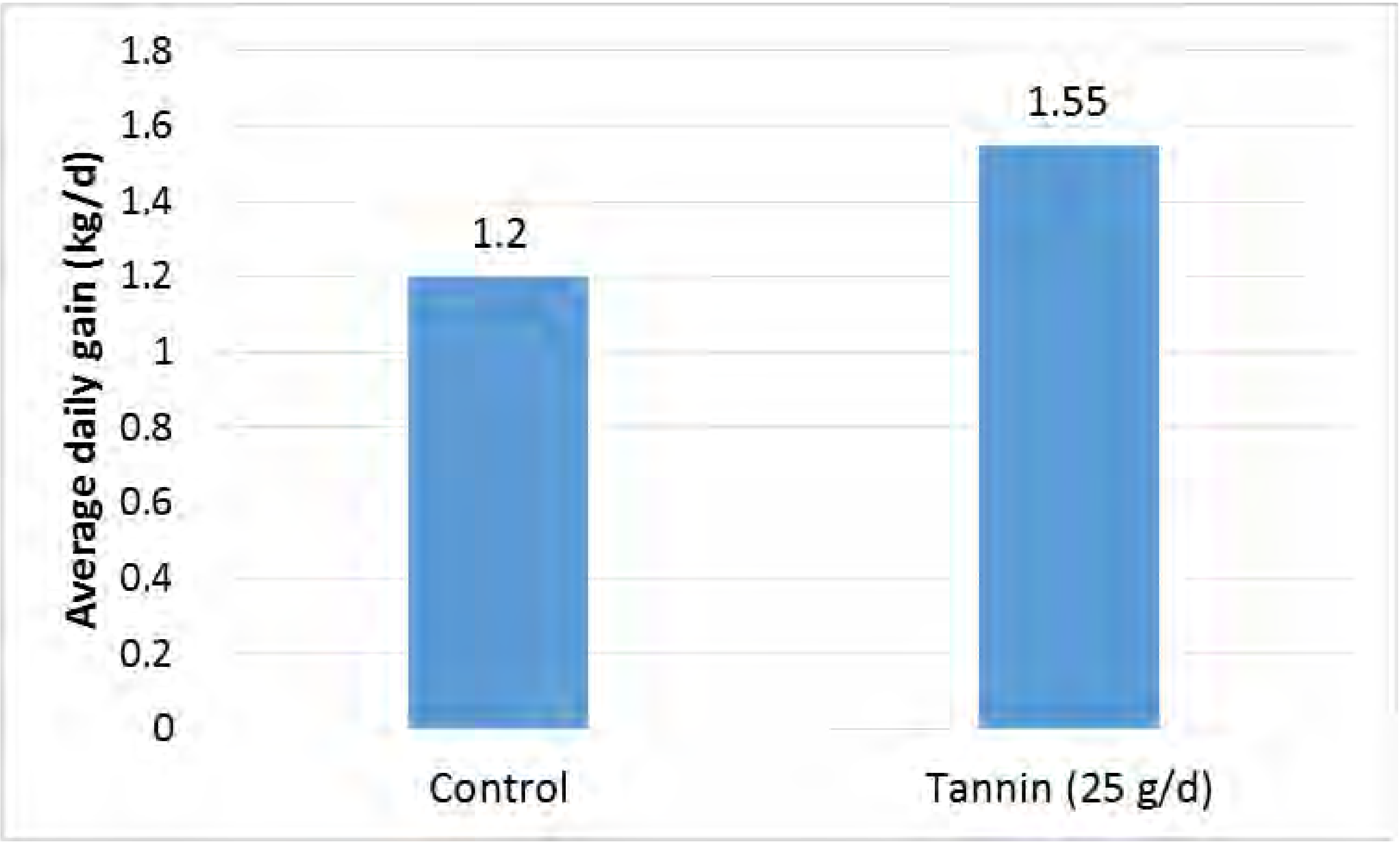
Tannin level = 15 g/kg DM

Trait[a]	Treatment			SEM	P-Value
	Control – CN (n = 12)	Chestnut – HT (n = 12)	Mimosa – CT (n = 12)		
IBW (kg)	412.3	414.4	409.1	12.6	0.95
FBW (kg)	481.3	478.5	479.2	14.2	0.99
ADG (kg)	1.89	1.77	1.92	0.20	0.64
Gain (kg)	79.27	74.43	80.44	8.45	0.64
DMI (kg/d)	12.17	11.40	11.26	0.96	0.50
DMI (g/kg BW)	27.7	26.1	25.8	1.80	0.44
Gain:feed	0.17	0.164	0.177	0.02	0.65
RFI (kg)	0.319	−0.452	0.132	0.58	0.62

[a] IBW: initial body weight; FBW: final 42-d body weight; ADG: average daily gain; DMI: dry matter intake; BW: body weight; RFI: residual feed intake.

Krueger et al. (2010)
Anim. Feed Sci. Technol. 159, 1-9

Feedlot trial on 40 beef cattle (Brahman cross)



Source: Jayanegara et al. (2016)
Unpublished study

Mode of action

CT level = 4 g/kg DM

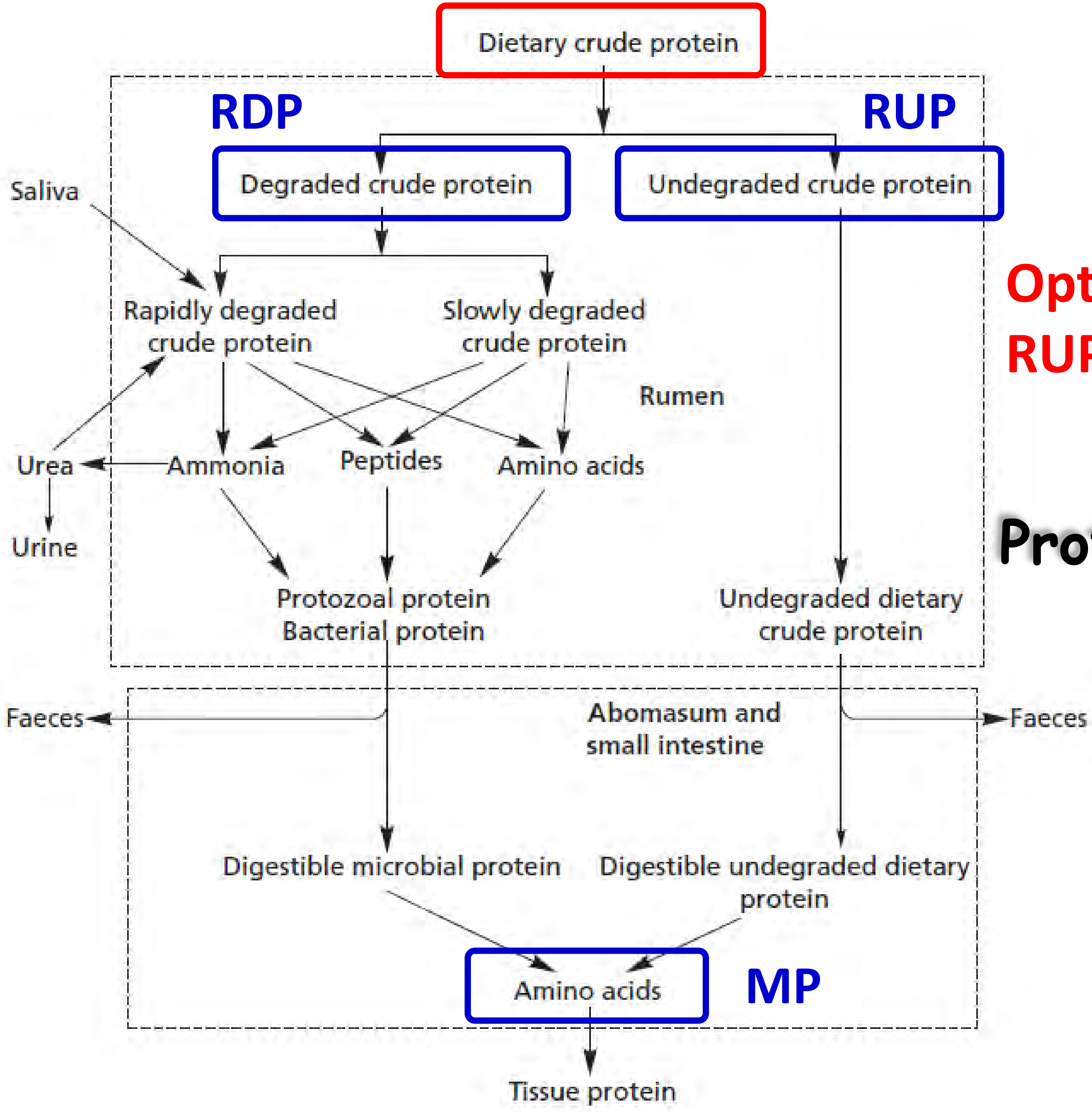
Table 5

Means and SEM of crude protein intake (CPI), rumen undegraded protein intake (RUPI), rumen degraded protein intake (RDPI), microbial protein synthesis (Pmic), availability of metabolizable protein (MP), levels of ruminal undegraded protein (RUP) and ruminal degraded protein (RDP), MP:CP ratio, and microbial efficiency (Mic. eff.), according to the experimental treatments.

Item	Without SBM		With SBM		*P*-value [a]		
	Without CT	With CT	Without CT	With CT	CT	SBM	CT × SBM
CPI	951.56 ± 49.15	961.94 ± 49.98	1160.83 ± 59.96	1143.28 ± 59.05	0.940	0.018	0.860
RUPI	232.26 ± 32.53	253.26 ± 35.47	302.24 ± 42.33	416.02 ± 58.26	0.161	0.033	0.093
RDPI	719.30 ± 32.55	708.68 ± 32.07	858.59 ± 38.85	727.26 ± 32.91	0.083	0.060	0.127
Pmic	491.63 ± 32.84	483.07 ± 32.27	466.31 ± 31.15	528.45 ± 35.30	0.447	0.771	0.324
MP	500.45 ± 24.30	511.78 ± 24.85	540.23 ± 26.23	671.03 ± 32.58	0.039	0.010	0.069
RUP (%) [b]	24.41 ± 3.21	26.33 ± 3.47	26.04 ± 3.43	36.39 ± 4.79	0.132	0.157	0.425
RDP (%) [b]	75.59 ± 3.98	73.67 ± 3.88	73.96 ± 3.89	63.61 ± 3.35	0.132	0.157	0.425
MP:CP	52.59 ± 1.04	53.20 ± 1.05	46.54 ± 0.92	58.69 ± 1.16	0.001	0.425	0.002
Mic. eff. [c]	93.64 ± 6.38	93.62 ± 6.38	94.59 ± 6.44	94.53 ± 6.44	0.936	0.807	0.786

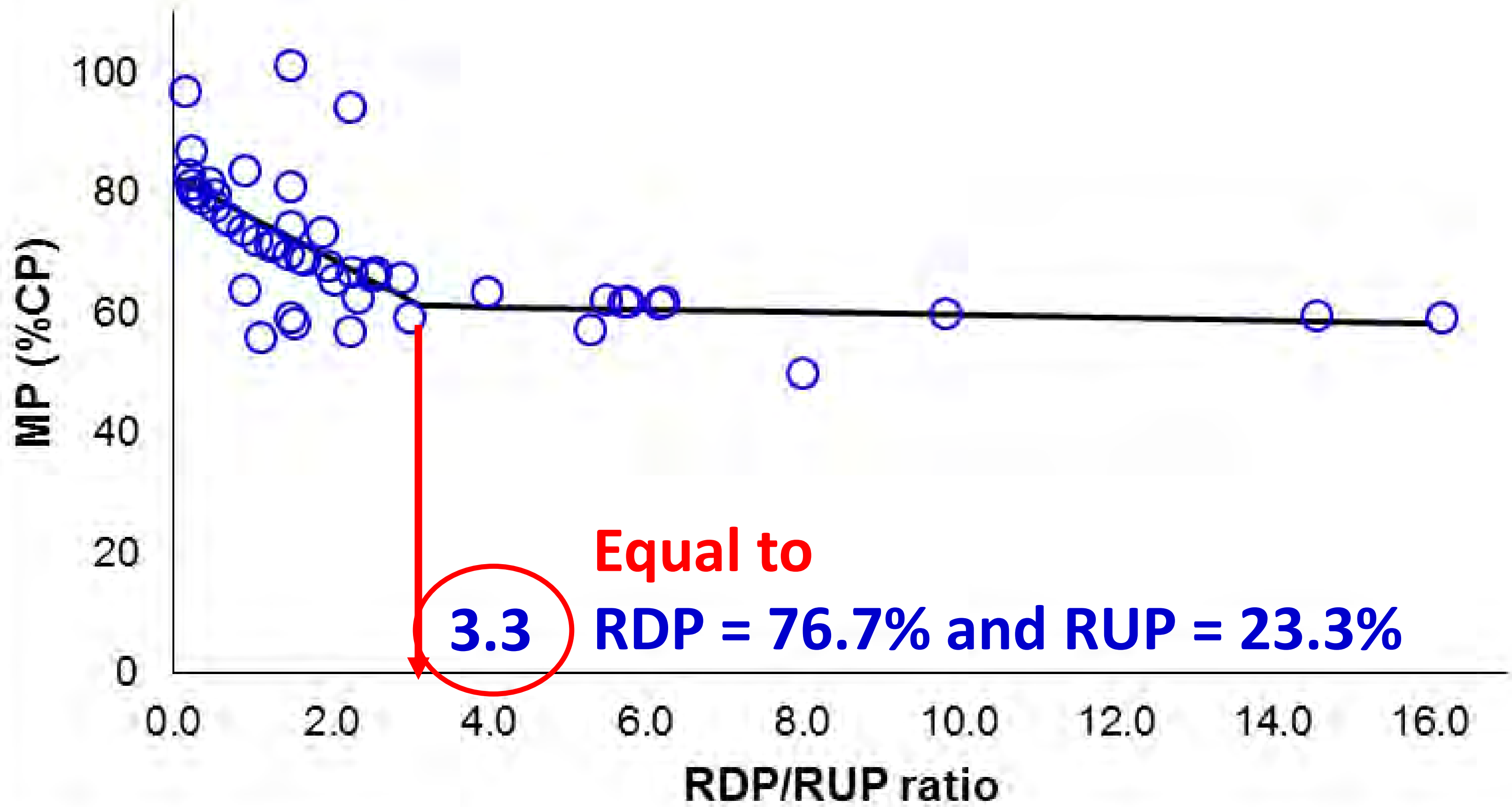
[a] CT indicates condensed tannin; SBM, soybean meal; CT × SBM, interaction between condensed tannin and soybean meal.
[b] Percent of crude protein.
[c] Gram per kilogram of TDN intake.

Mezzomo et al. (2011)
Livest. Sci. 141, 1-11



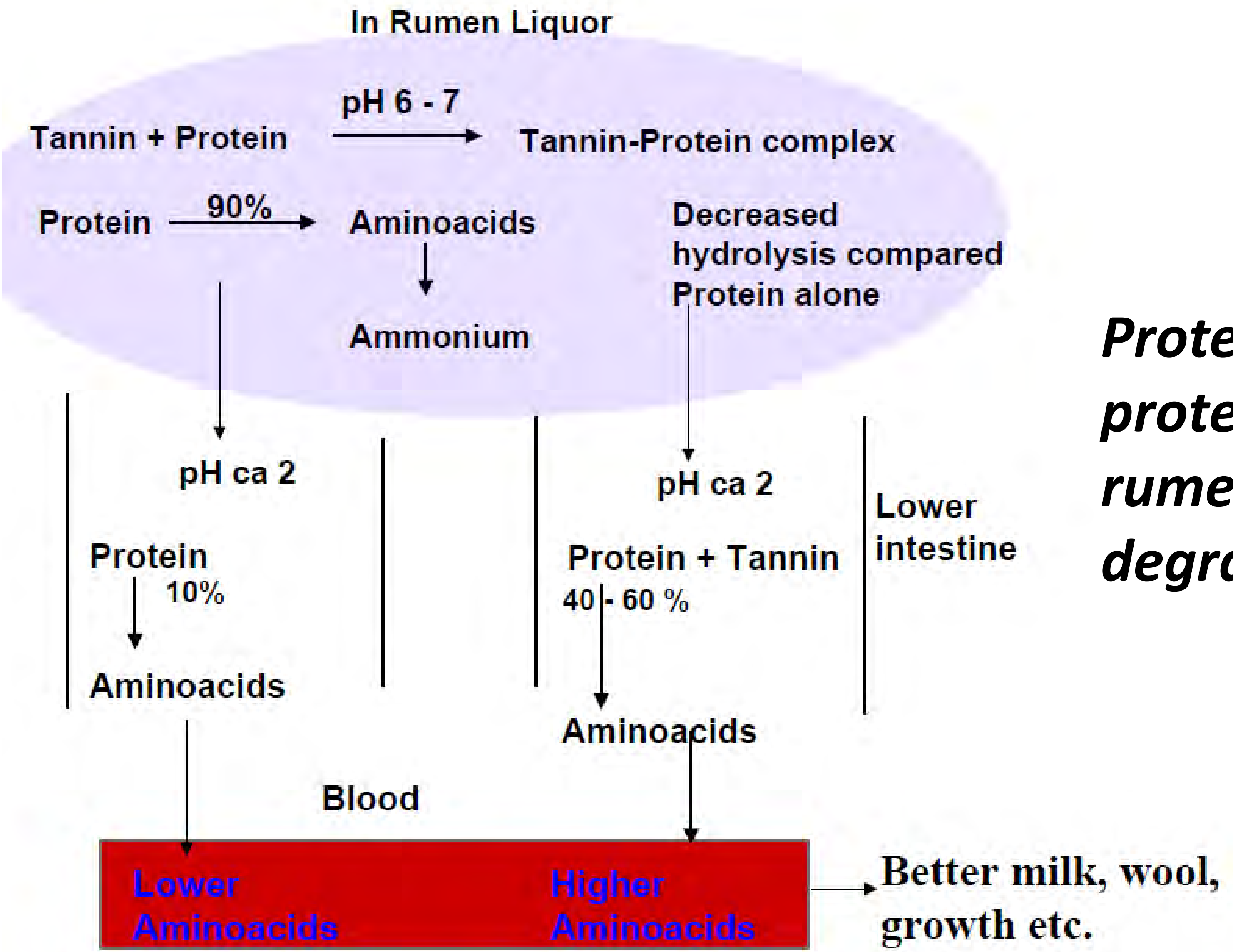
Optimum RDP to RUP ratio?

Protein partition



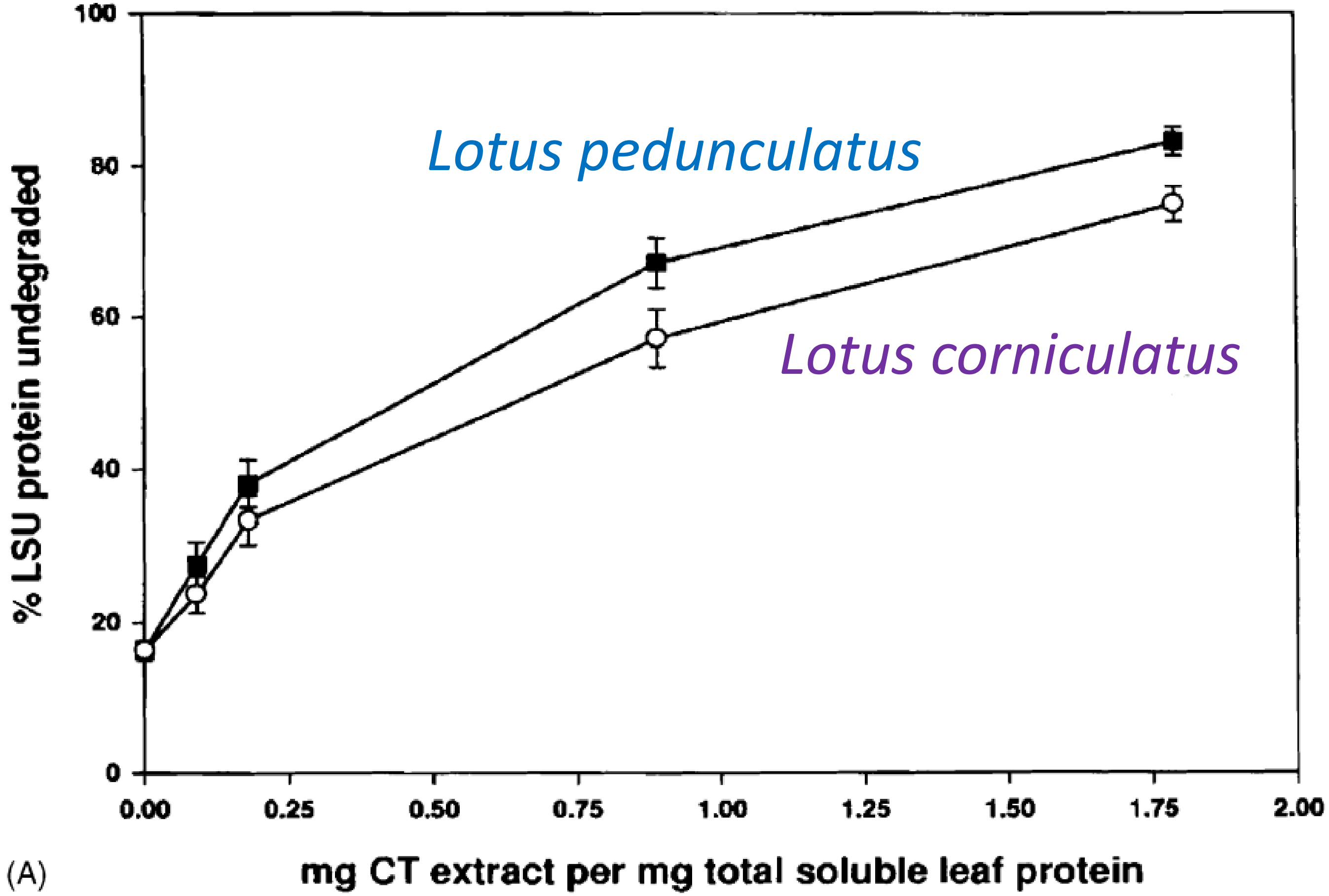
Jayanegara et al. (2016)

Proc. 3rd APIS & 3rd ARCAP 170-173



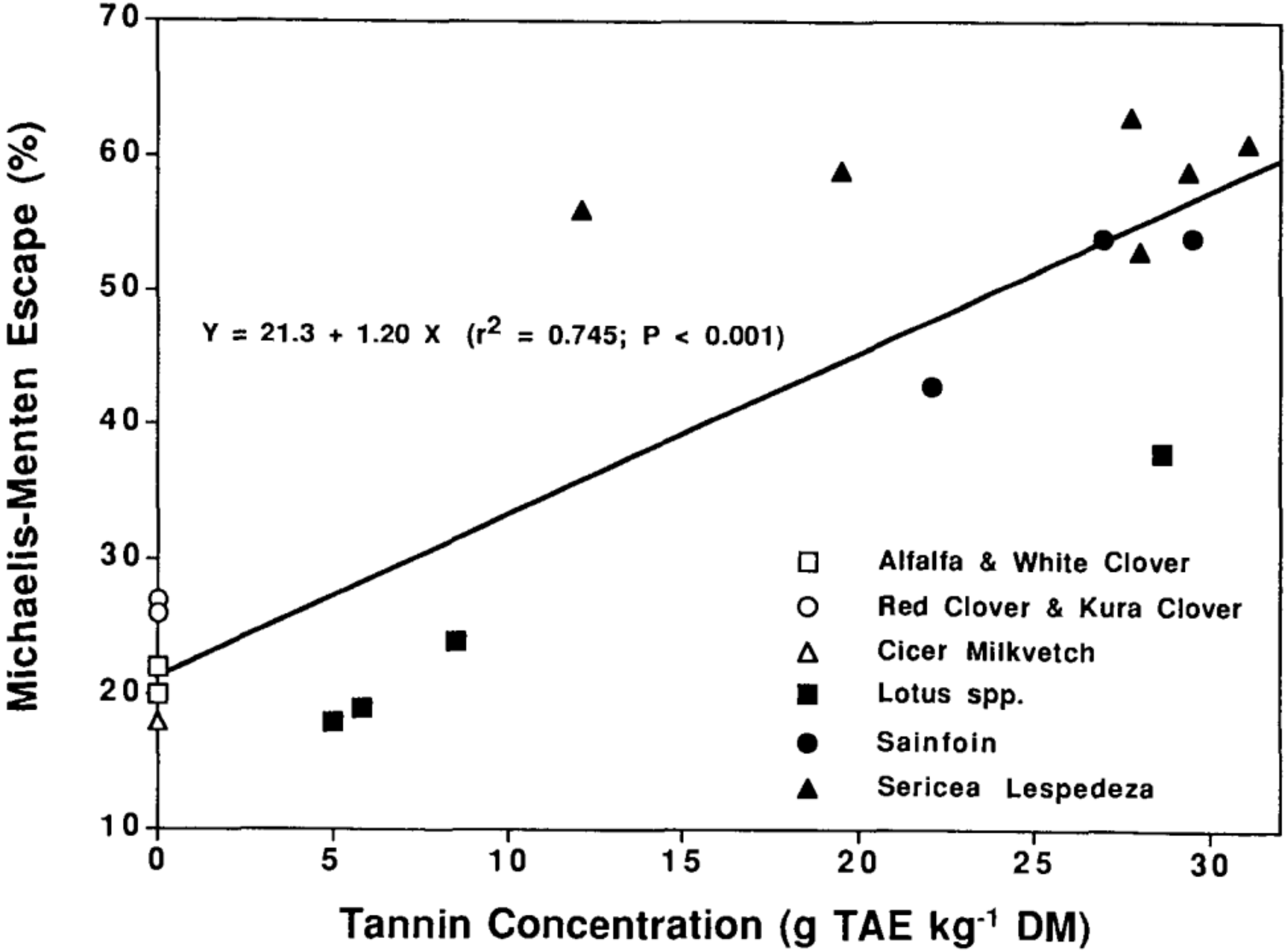
Protection of protein from rumen degradation

Rubisco protein (from white clover)

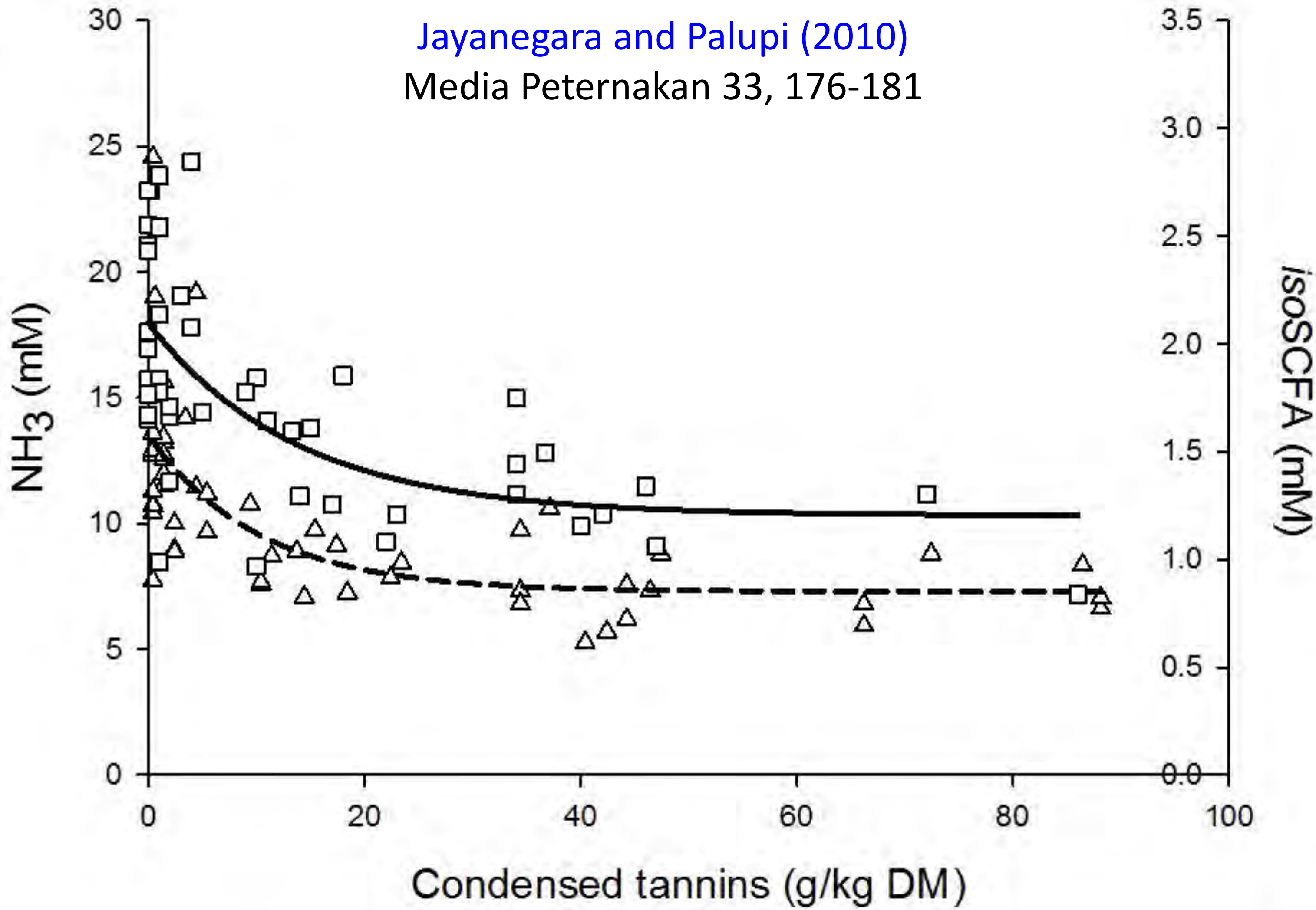


Source: Min et al. (2003)
Anim. Feed Sci. Technol. 106, 3-19

Ruminal protein escape



Source: Broderick and Albrecht (1997)
Crop Sci. 37, 1884-1891


Jayanegara and Palupi (2010)
Media Peternakan 33, 176-181
NH_3 (mM)
isoSCFA (mM)
Condensed tannins (g/kg DM)
0
5
10
15
20
25
30
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
20
40
60
80
100

Control of gastro-intestinal parasites

Dairy goat:

Decreased nematode infection → improved production

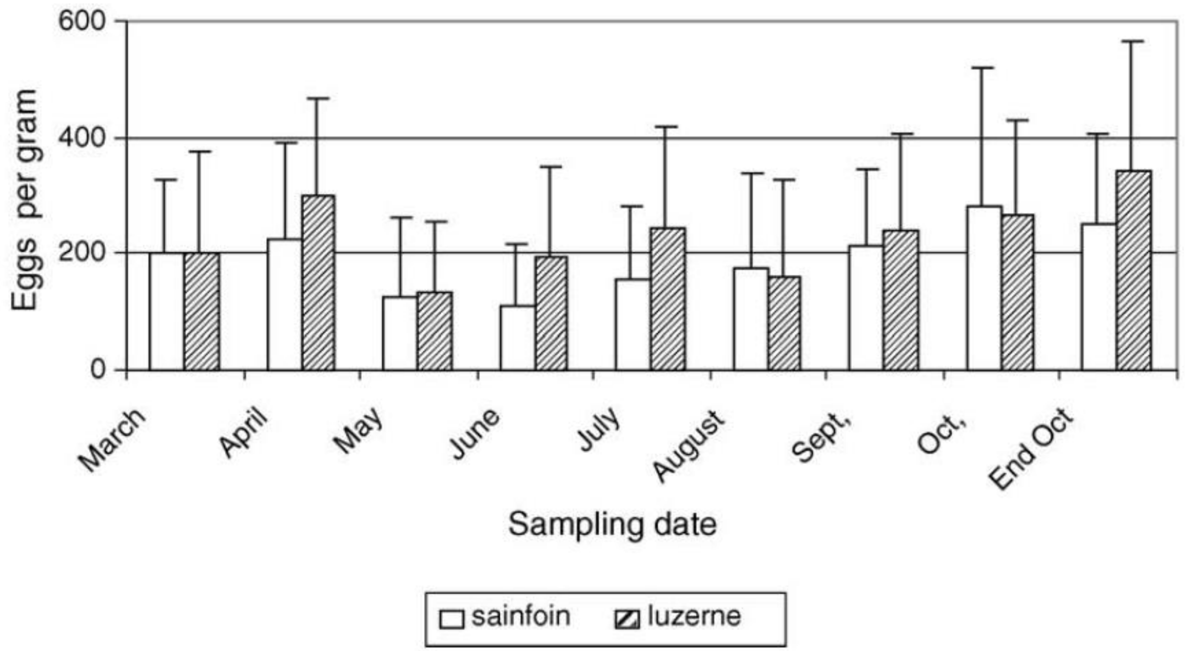


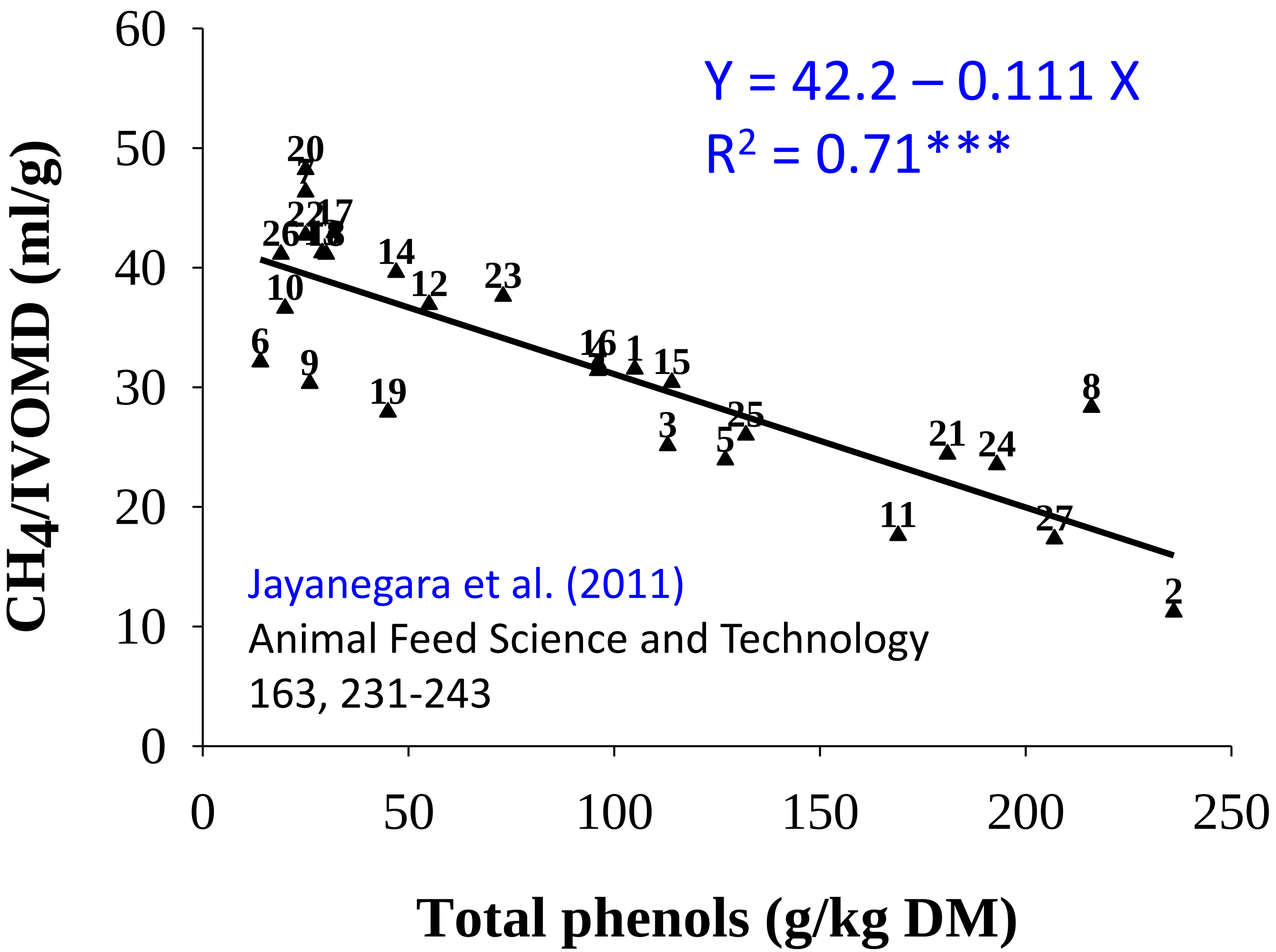
Fig. 1. Comparison of faecal egg counts (mean values + S.D.) in goats receiving or not sainfoin hay. The samples were performed at the start of the experimental period of study and after each 10-day period of hay distribution. The statistical analysis of variance on repeated measurements have shown a significant difference between the two groups ($P < 0.05$).

Hoste et al. (2005)
Small Rum. Res. 59, 265-271

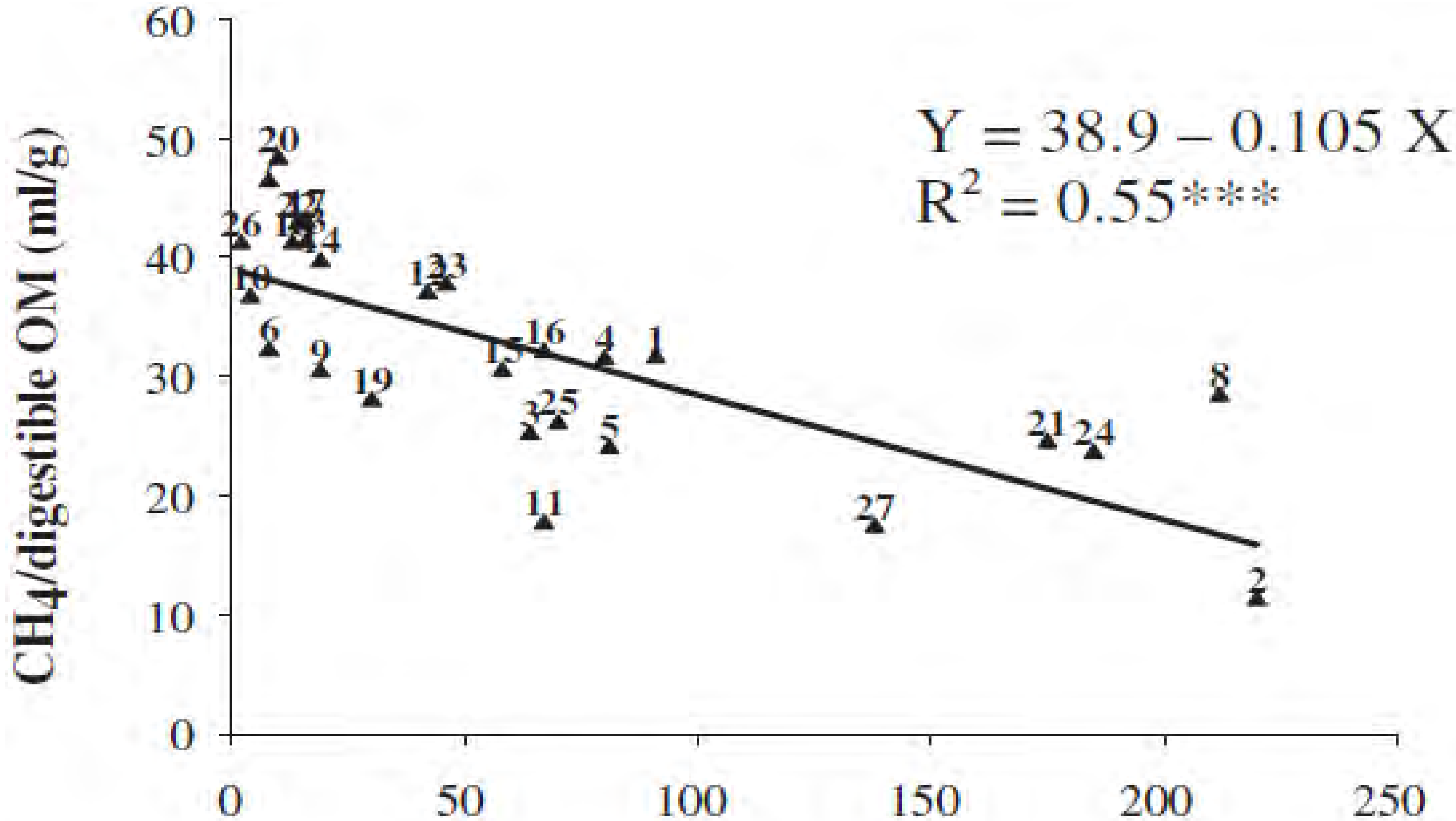
D. Effects on methane emissions

(1) Polyphenol-containing plants

27 tropical forage species

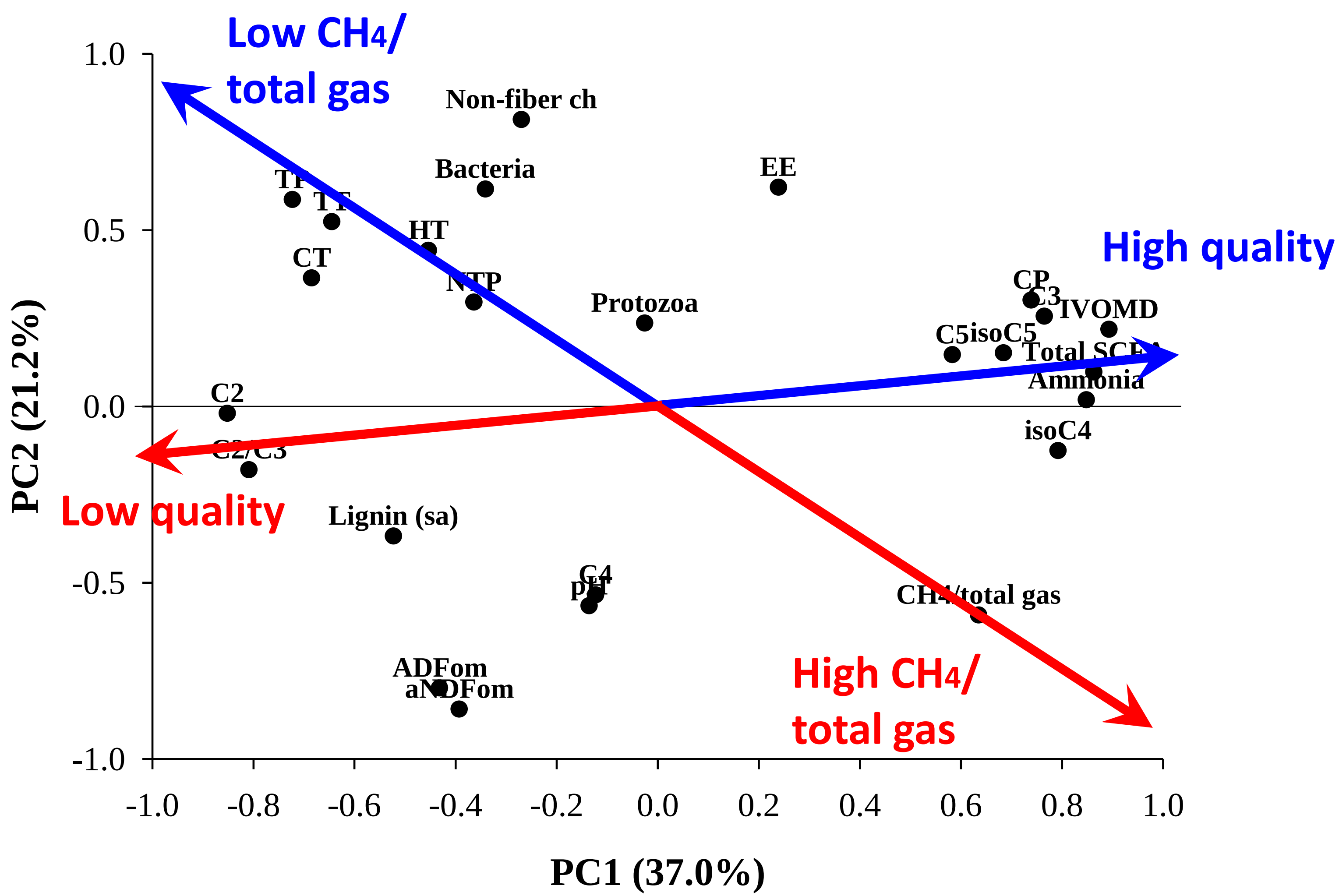


Jayanegara et al. (2011)
Animal Feed Science and Technology
163, 231-243

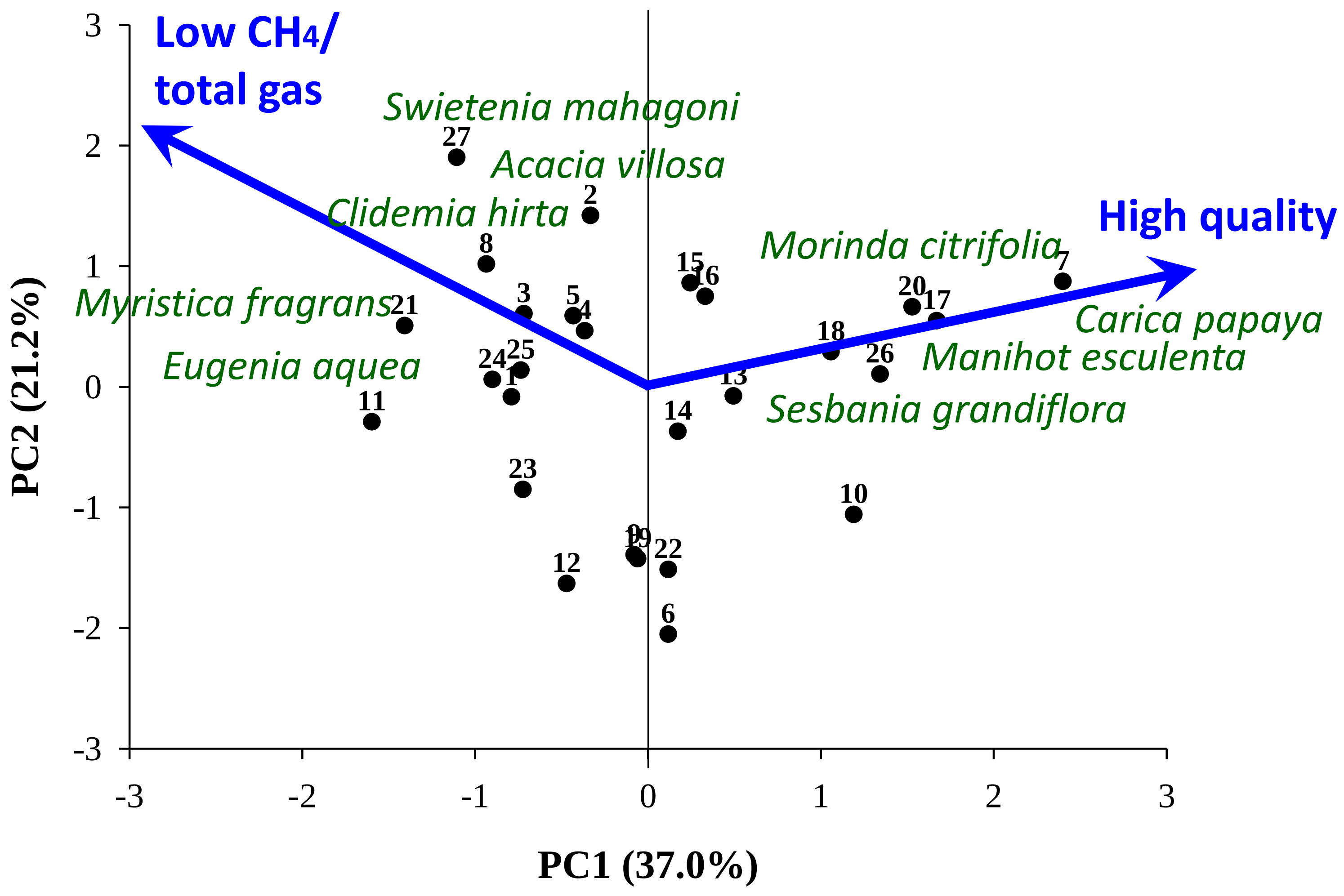


Total tannins (g/kg DM)

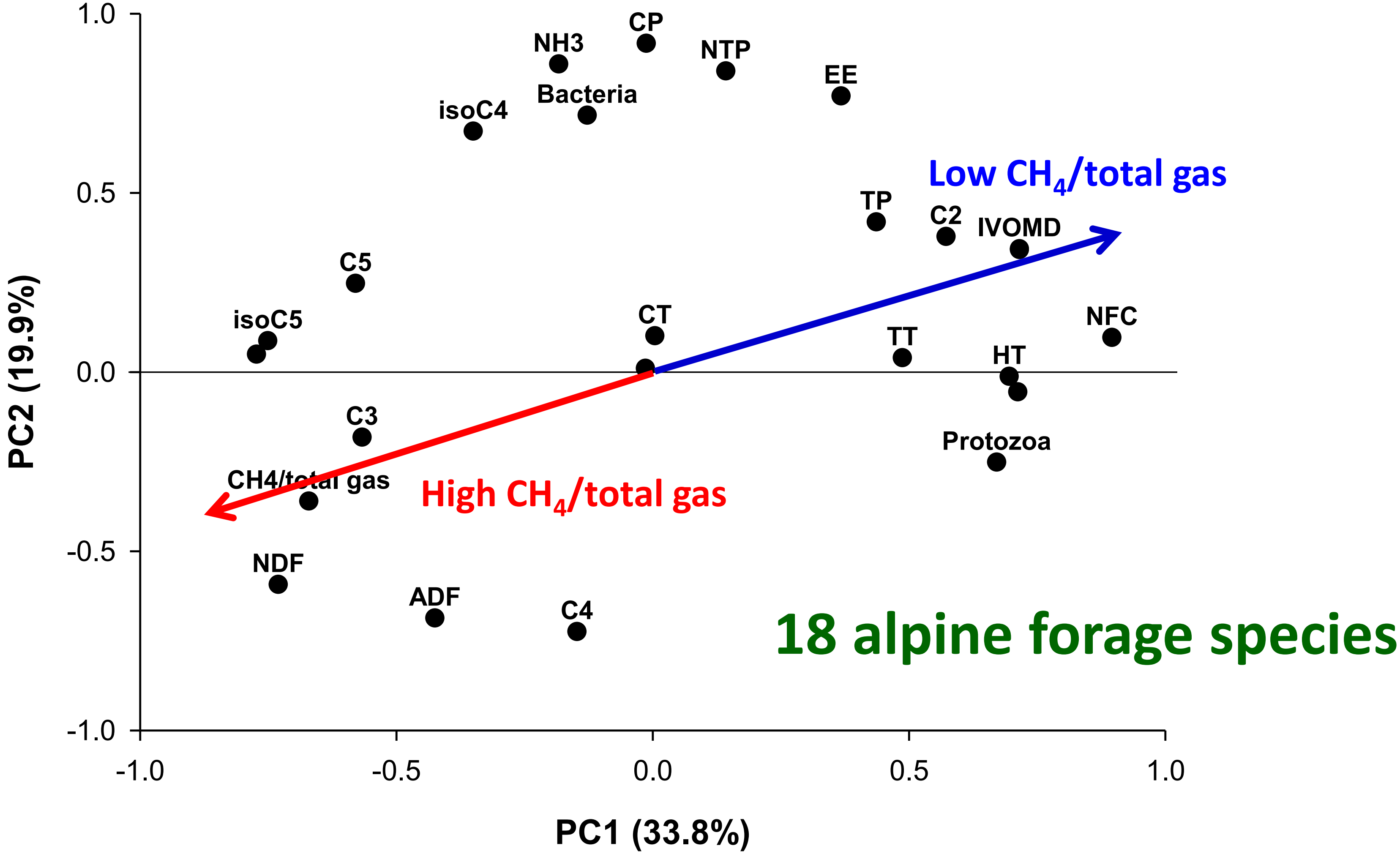
Jayanegara et al. (2011)
Animal Feed Science and Technology
163, 231-243



Jayanegara et al. (2011)
Animal Feed Science and Technology
163, 231-243

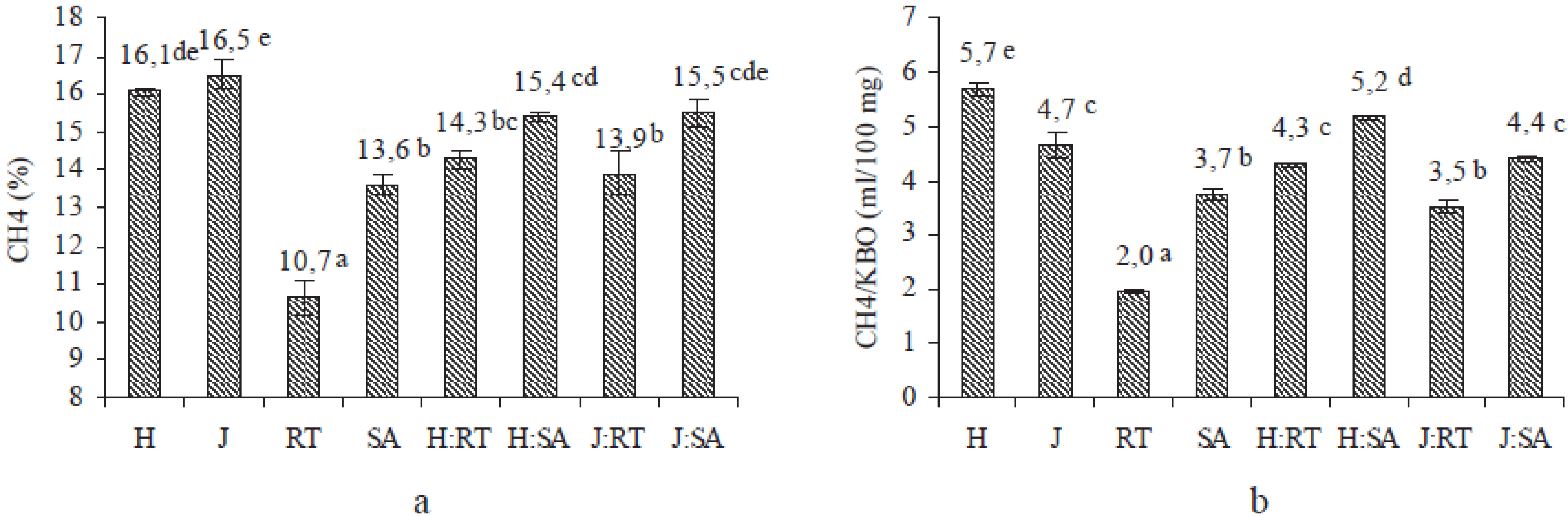


Source: Jayanegara et al. (2011)
J. Sci. Food Agric. 91, 1863-1870



(2) Mixture of polyphenol-containing plants

Total tannins (%DM): RT = 20.9, SA = 3.6



Gambar 1. Produksi metana/CH_4 (a) dan produksi metana per unit bahan organik tercerna (b) dari ransum perlakuan. KBO = kecernaan bahan organik; H = hay; J = jerami; RT = *Rhus typhina*; SA = *Salix alba*; H:RT = Hay:*R. typhina* 70:30 (w/w); H:SA = Hay:*S. alba* 70:30 (w/w); J:RT = Jerami:*R. typhina* 70:30 (w/w); J:SA = Jerami:*S. alba* 70:30 (w/w). Superskrip berbeda menunjukkan berbeda nyata (P<0,01).

Jayanegara et al. (2009)
Media Peternakan 32, 120-129

Tabel 4. Matriks korelasi (r) antara komposisi kimia pakan dan peubah fermentasi rumen

Peubah	Gas	a + b	c	KBO	CH_4	CH_4/KBO
Protein kasar	0,81*	0,44tn	0,89**	0,98***	-0,55tn	-0,26tn
Lemak kasar	-0,15tn	-0,65tn	0,57tn	0,49tn	-0,88**	-0,79*
NDF	-0,57tn	-0,00tn	-0,93***	-0,98***	0,81*	0,55tn
ADF	-0,72*	-0,23tn	-0,94***	-0,99***	0,68tn	0,39tn
Hemiselulosa	-0,15tn	0,46tn	-0,77*	-0,80*	0,97***	0,84**
Total fenol	-0,13tn	-0,66tn	0,60tn	0,60tn	-0,97***	-0,92***
Total tanin	-0,18tn	-0,69tn	0,56tn	0,54tn	-0,95***	-0,90**
Tanin terkondensasi	0,53tn	0,45tn	0,31tn	0,54tn	-0,12tn	-0,08tn

Keterangan: KBO=kecernaan bahan organik; CH_4=metana; a + b=produksi gas maksimum pada t mendekati tak hingga (asimtot); c=laju produksi gas kumulatif; tn=tidak berbeda nyata; *=berbeda nyata pada P<0,05; **=berbeda nyata pada P<0,01; ***=berbeda nyata pada P<0,001; NDF=*neutral detergent fibre*; ADF=*acid detergent fibre*.

Jayanegara et al. (2009)
Media Peternakan 32, 120-129

(3) Associative/interaction effects

Chemical composition of the plants (g/kg DM)

Treatment	CP	EE	NDF	ADF	ADL	TP	NTP	TT	CT	HT
Carica papaya	386	47	155	135	46	25	17	8	0	8
Clidemia hirta	129	22	232	181	69	216	4	212	10	202
Swietenia mahagoni	112	45	281	222	78	207	69	138	86	52
Eugenia aquea	100	35	479	382	181	169	102	67	40	27

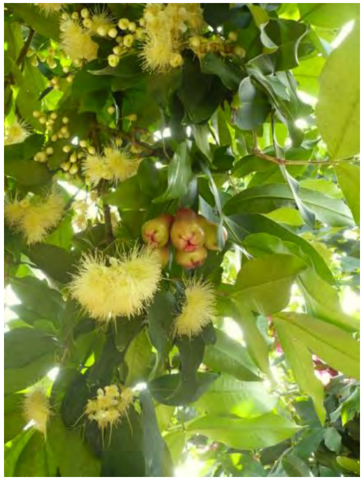
CP, crude protein; **EE**, ether extract; **NDF**, neutral detergent fiber;
ADF, acid detergent fiber; **ADL**, acid detergent lignin;
TP, total phenols; **NTP**, non-tannin phenols; **TT**, total tannins;
CT, condensed tannins; **HT**, hydrolysable tannins.

Jayanegara et al. (2013)
British Journal of Nutrition 109, 615-622

Carica papaya

Swietenia mahagoni

Eugenia aquea



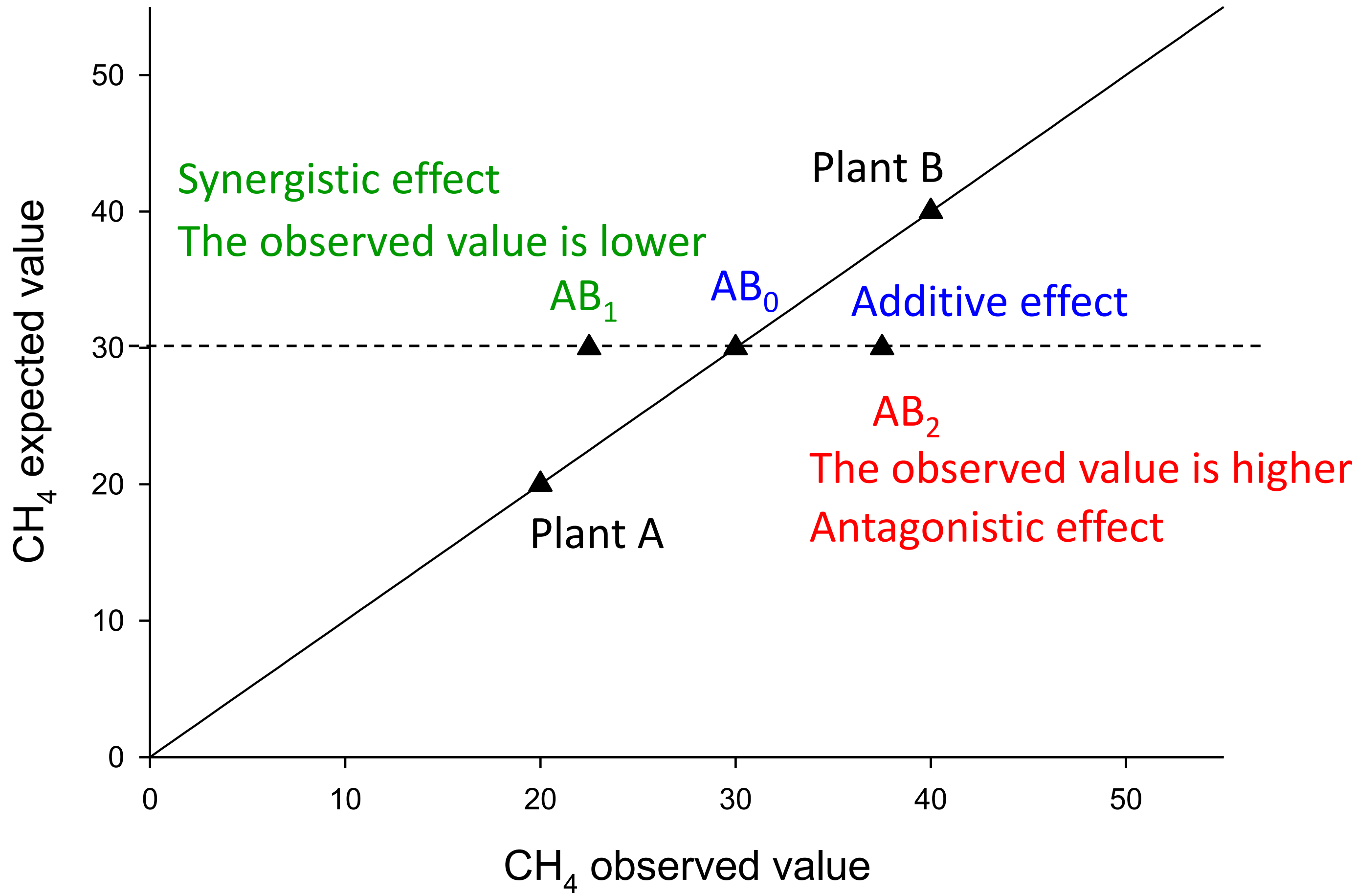
Clidemia hirta

Jayanegara et al. (2013)
British Journal of Nutrition 109, 615-622

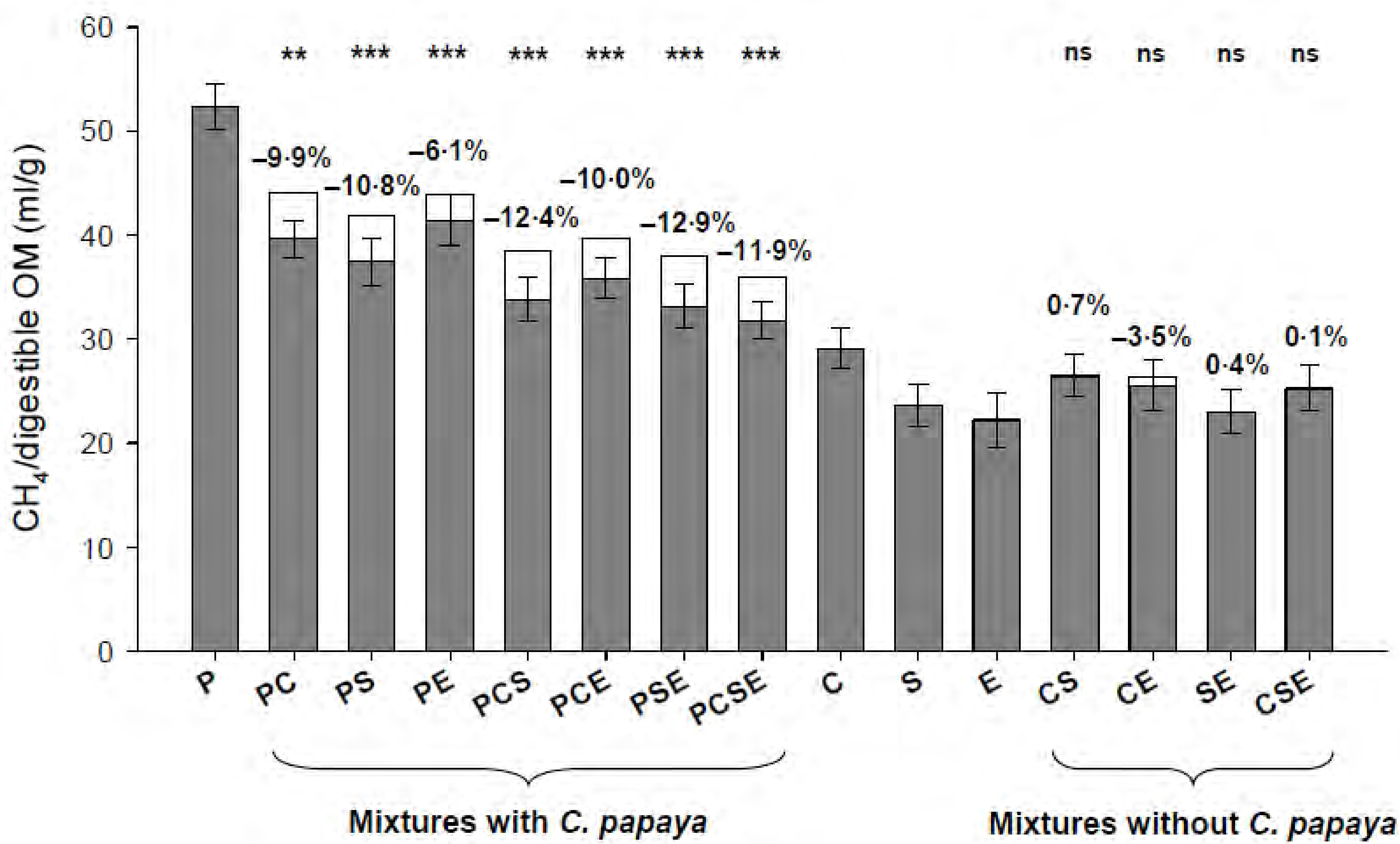
Structure of the treatments (as mg DM of plants incubated)

No.	Treatment	*Carica papaya*	*Clidemia hirta*	*Swietenia mahagoni*	*Eugenia aquea*	
1	Cp	200				Single plants
2	Ch		200			
3	Sm			200		
4	Ea				200	
5	CpCh	100	100			2 plants
6	CpSm	100		100		
7	CpEa	100			100	
8	ChSm		100	100		
9	ChEa		100		100	
10	SmEa			100	100	
11	CpChSm	67	67	67		3 plants
12	CpChEa	67	67		67	
13	CpSmEa	67		67	67	
14	ChSmEa		67	67	67	
15	CpChSmEa	50	50	50	50	4 plants

Associative effect (%) = 100 x (observed–expected)/expected

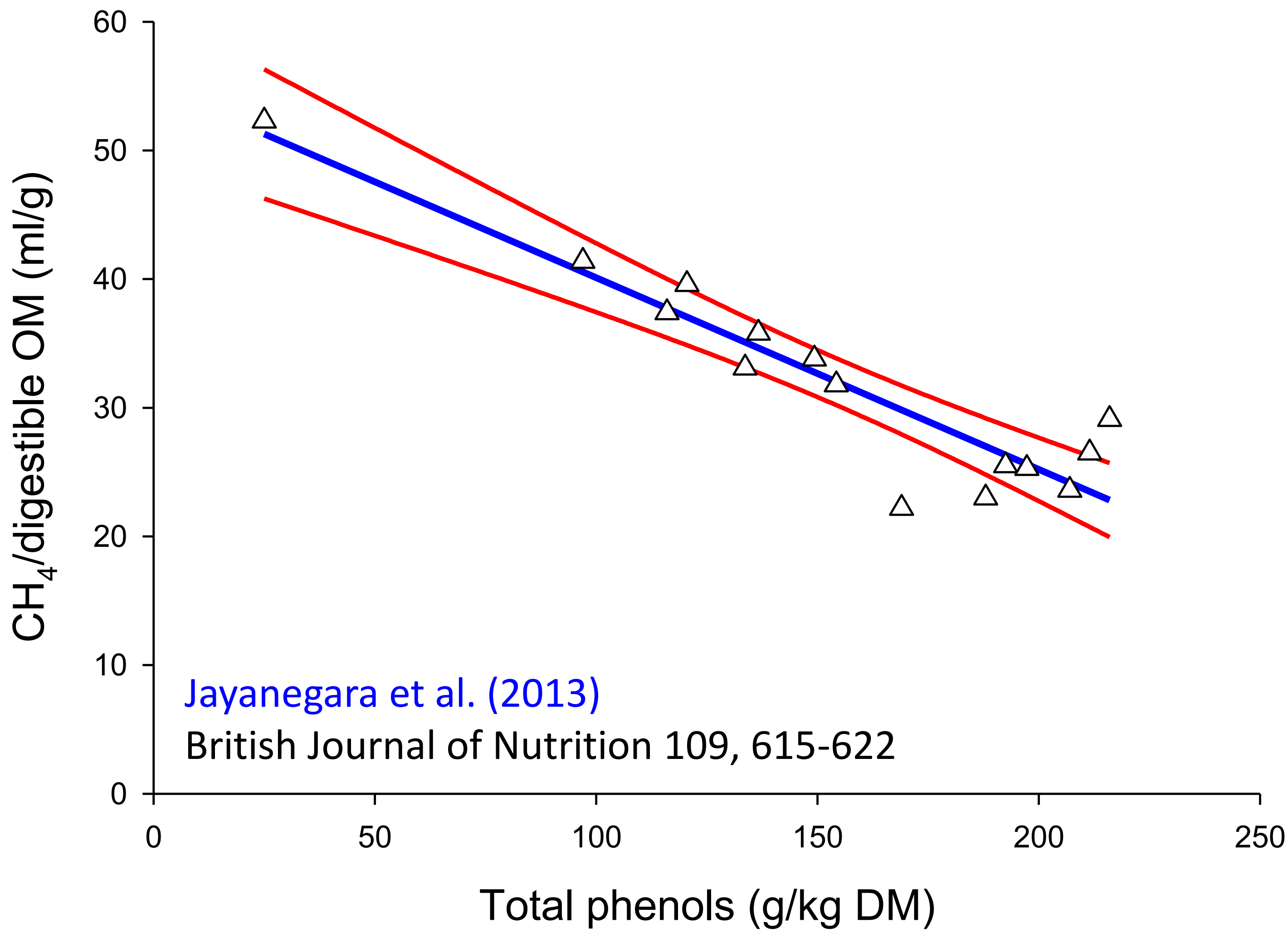


Assessment of the associative effects



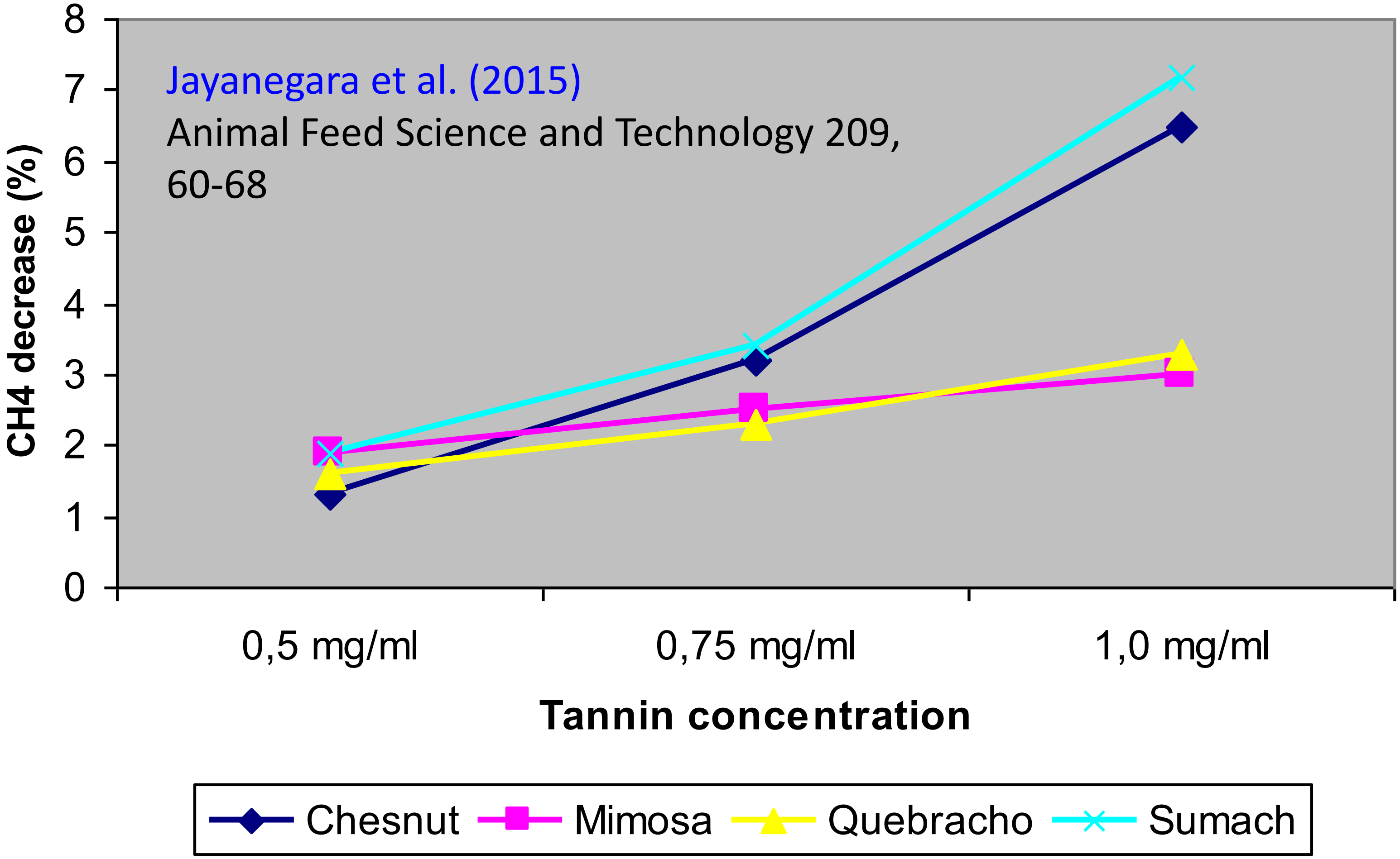
Jayanegara et al. (2013)

British Journal of Nutrition 109, 615-622

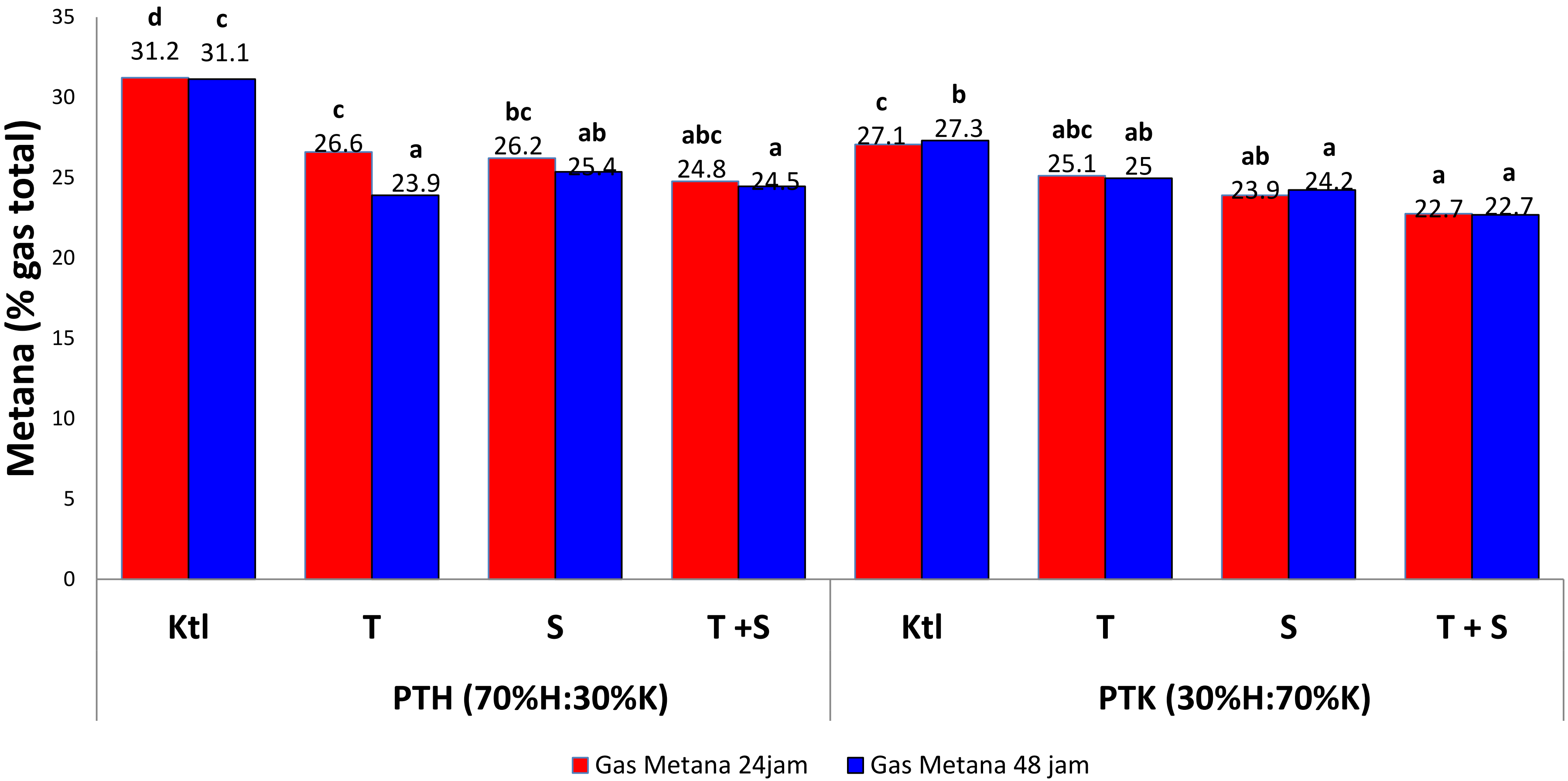

60
50
40
30
20
10
0
0
50
100
150
200
250
CH4/digestible OM (ml/g)
Total phenols (g/kg DM)
Jayanegara et al. (2013)
British Journal of Nutrition 109, 615-622

(4) Tannin extracts

- ↓ methanogen
- ↓ protozoa



Source: Yogianto et al. (2014)
J. Indon. Trop. Anim. Agric. 39, 144-151



Ktl= kontrol; T= tanin (2mg/ml); S=Saponin (2mg/ml); T+S= kombinasi tanin dan saponin (1mg/ml+1mg/ml); PTH=pakan tinggi hijauan; PTK=pakan tinggi konsentrat; H= hijauan; K= konsentrat.

Source: Yuliana et al. (2014)
J. Indon. Trop. Anim. Agric. 39, 91-97

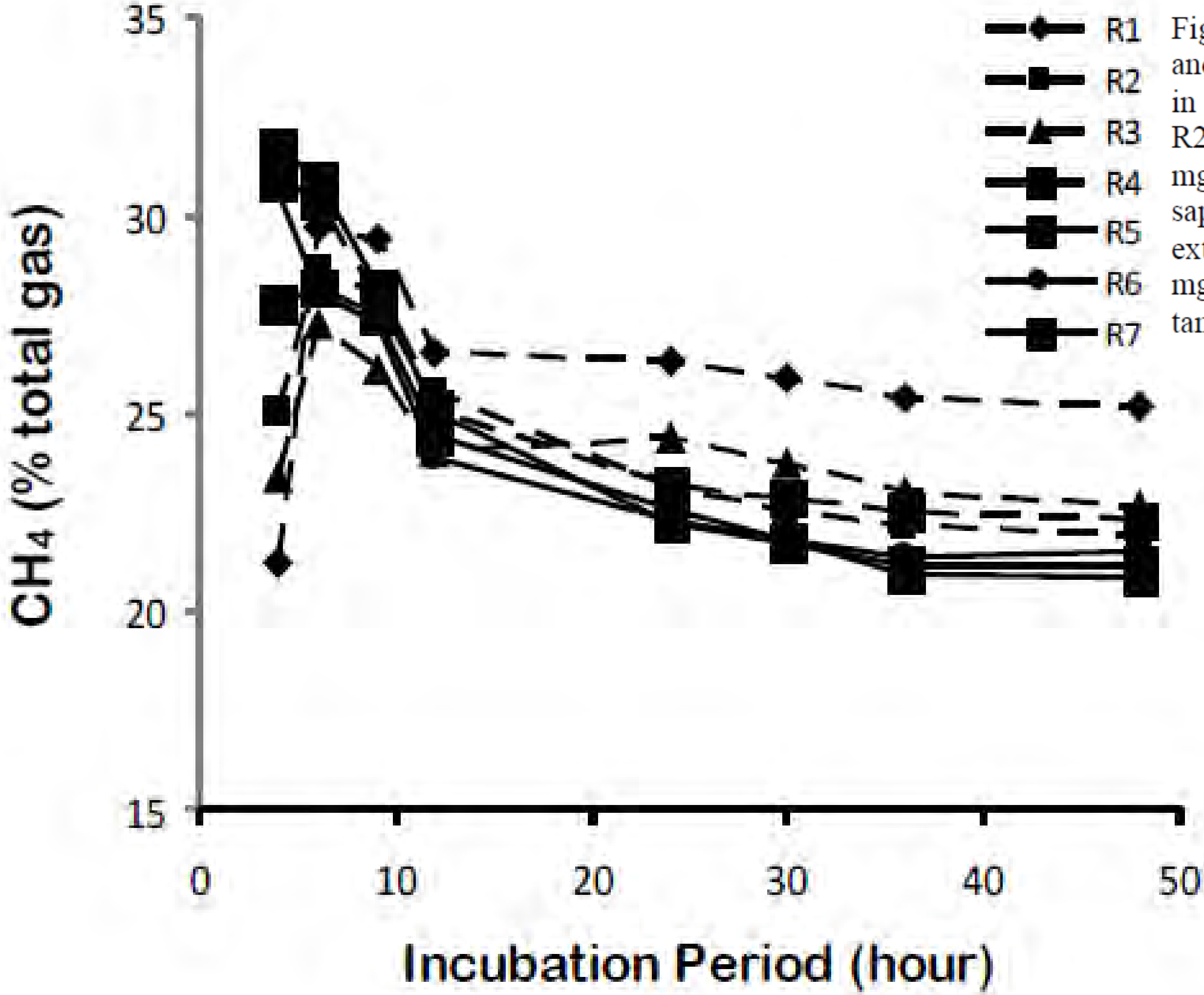
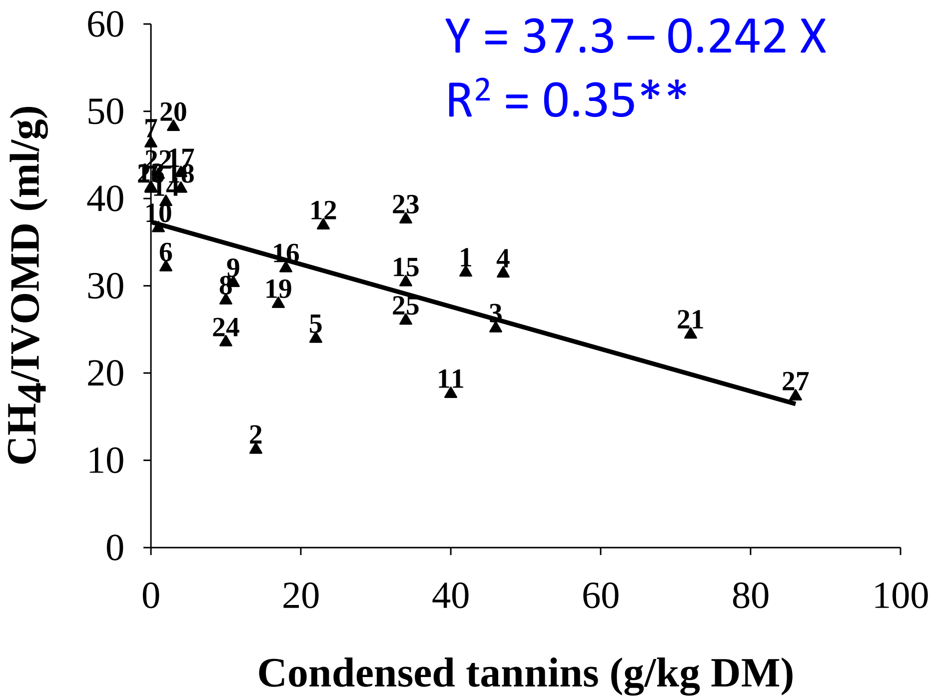
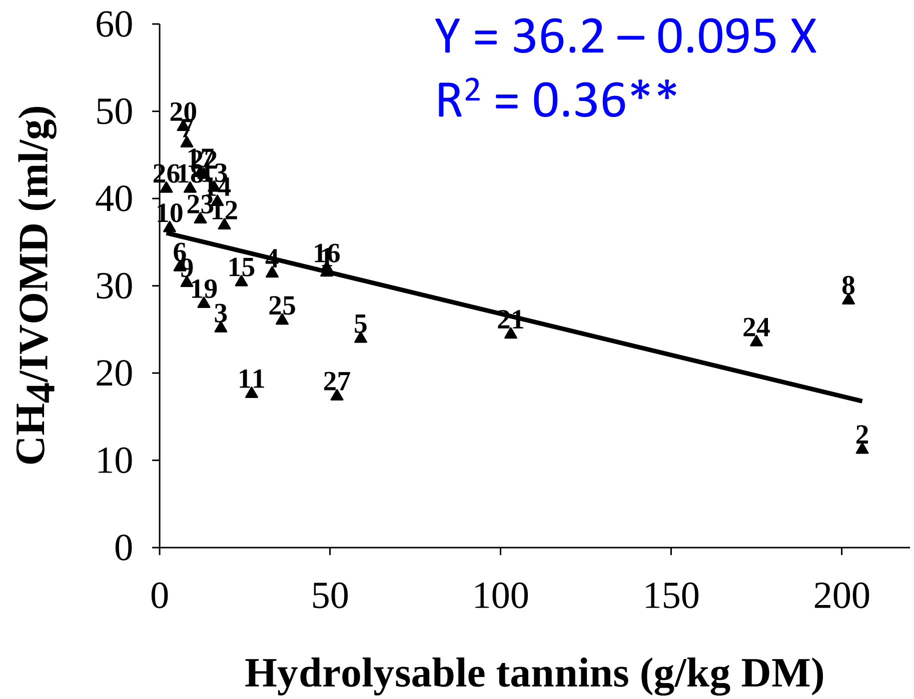


Figure 2. Methane Concentration of Added Tannin and Saponin Extracts in Subtrate when Incubated in Buffered-rumen Fluid. R1: control substrate, R2: R1 + 0.5 mg/mL tannin extract, R3: R1 + 1 mg/mL tannin extract, R4: R1 + 0.5 mg/mL saponin extract, R5: R1 + 1 mg/mL saponin extract, R6: R1 + 0.5 mg/mL tannin extract + 0.5 mg/mL saponin extract, and R7: R1 + 1 mg/mL tannin extract + 1 mg/mL saponin extract.

(5) CT vs HT

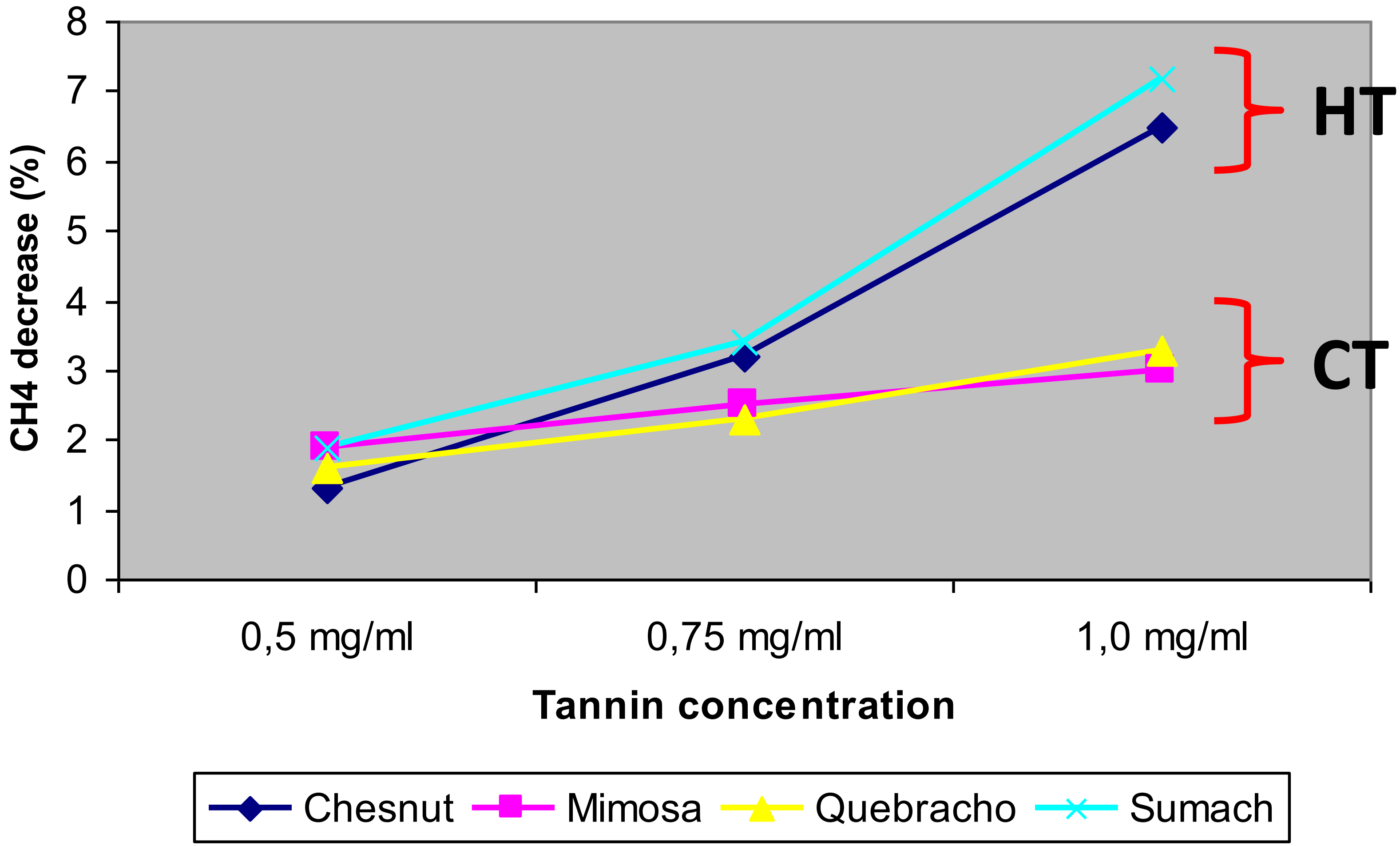




Jayanegara et al. (2011)

Animal Feed Science and Technology 163, 231-243

Jayanegara et al. (2015)
Animal Feed Science and Technology 209, 60-68



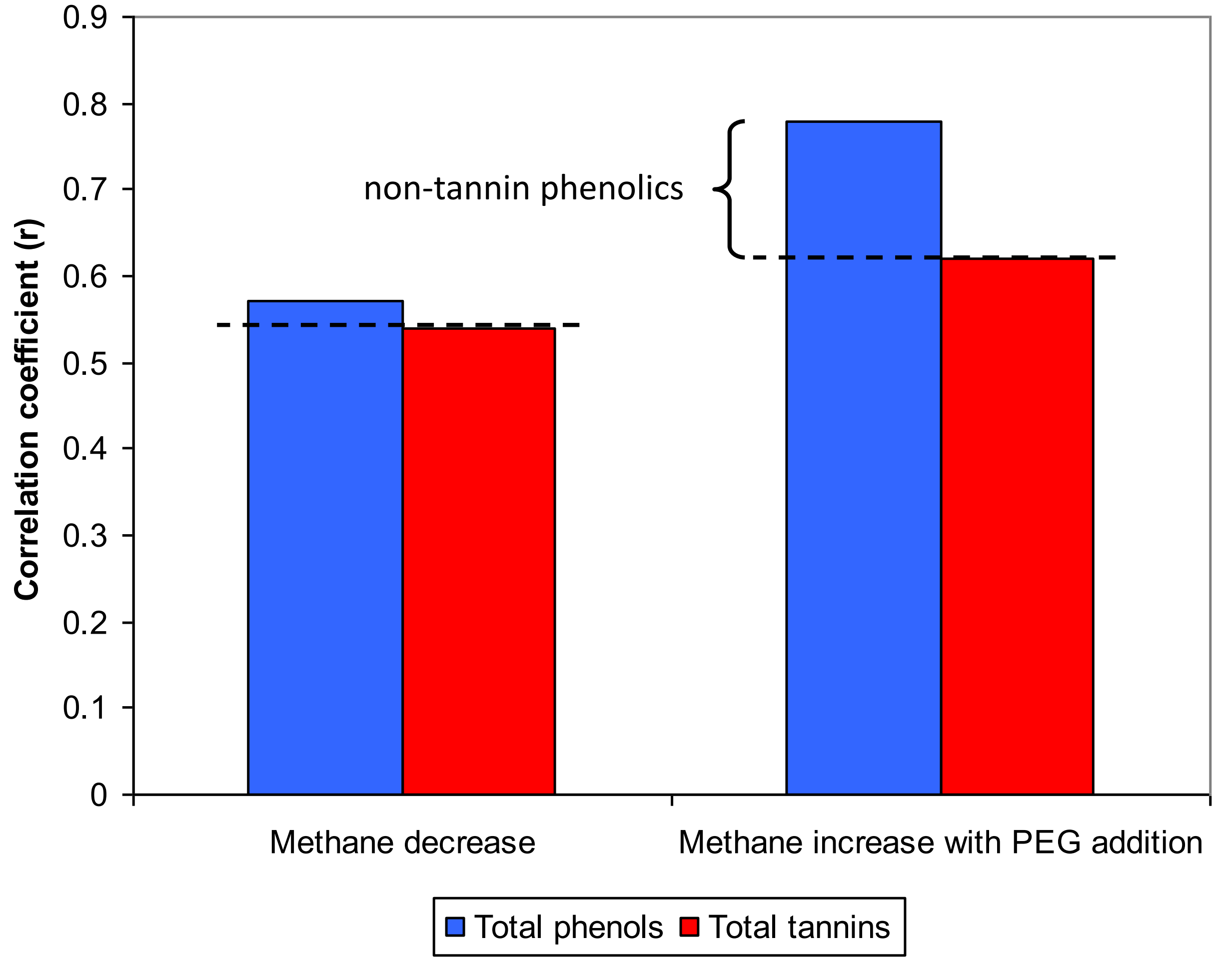
(6) Non-tannin phenols

Correlation between total phenols and methane reduction was higher than that between total tannins and methane reduction

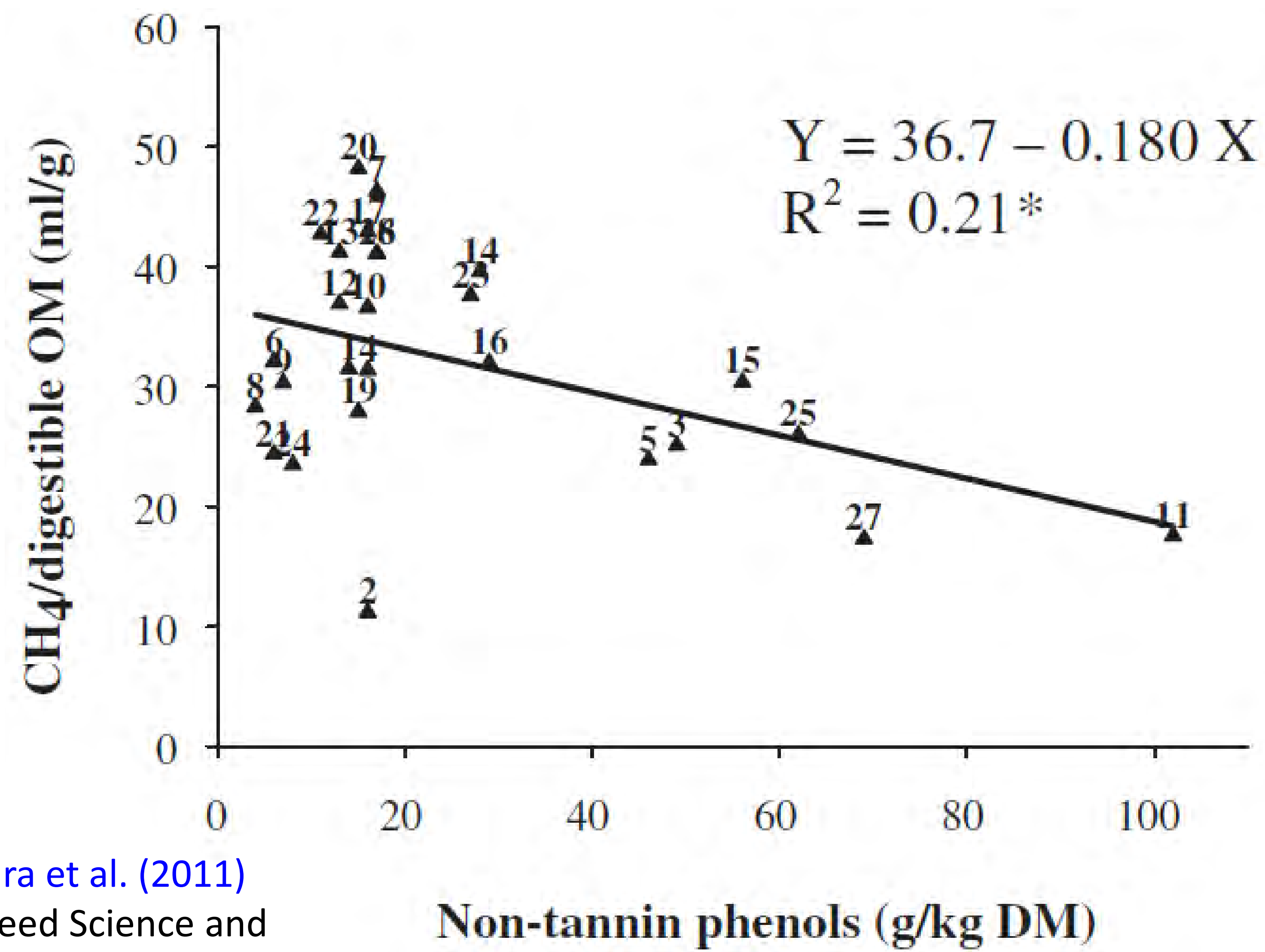
r-value **TP** > **TT**

(n = 17)

The role of non-tannin phenolics to decrease methane production??

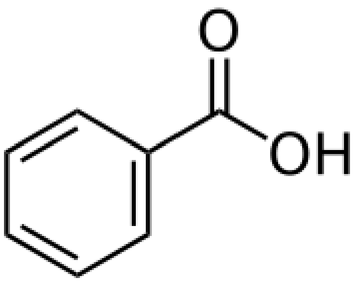


Jayanegara et al. (2009)
Animal Feed Science and Technology 150, 230-237

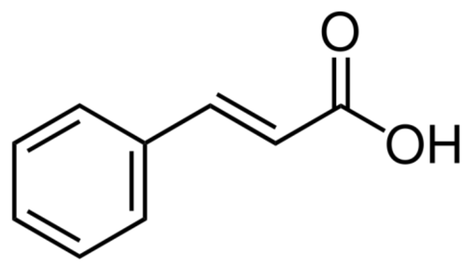


Jayanegara et al. (2011)
Animal Feed Science and Technology 163, 231-243

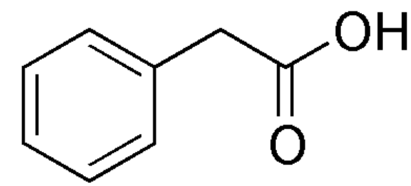
Jayanegara et al. (2009)
Media Peternakan 32, 53-62



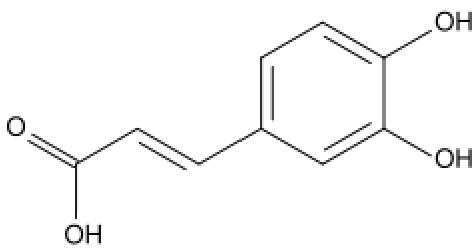
Benzoic acid



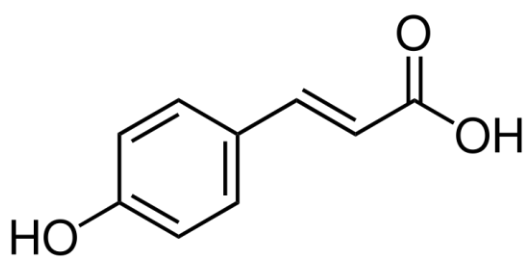
Cinnamic acid



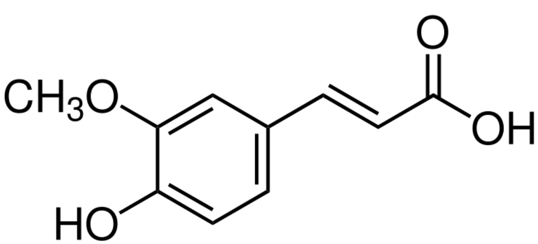
Phenylacetic acid



Caffeic acid

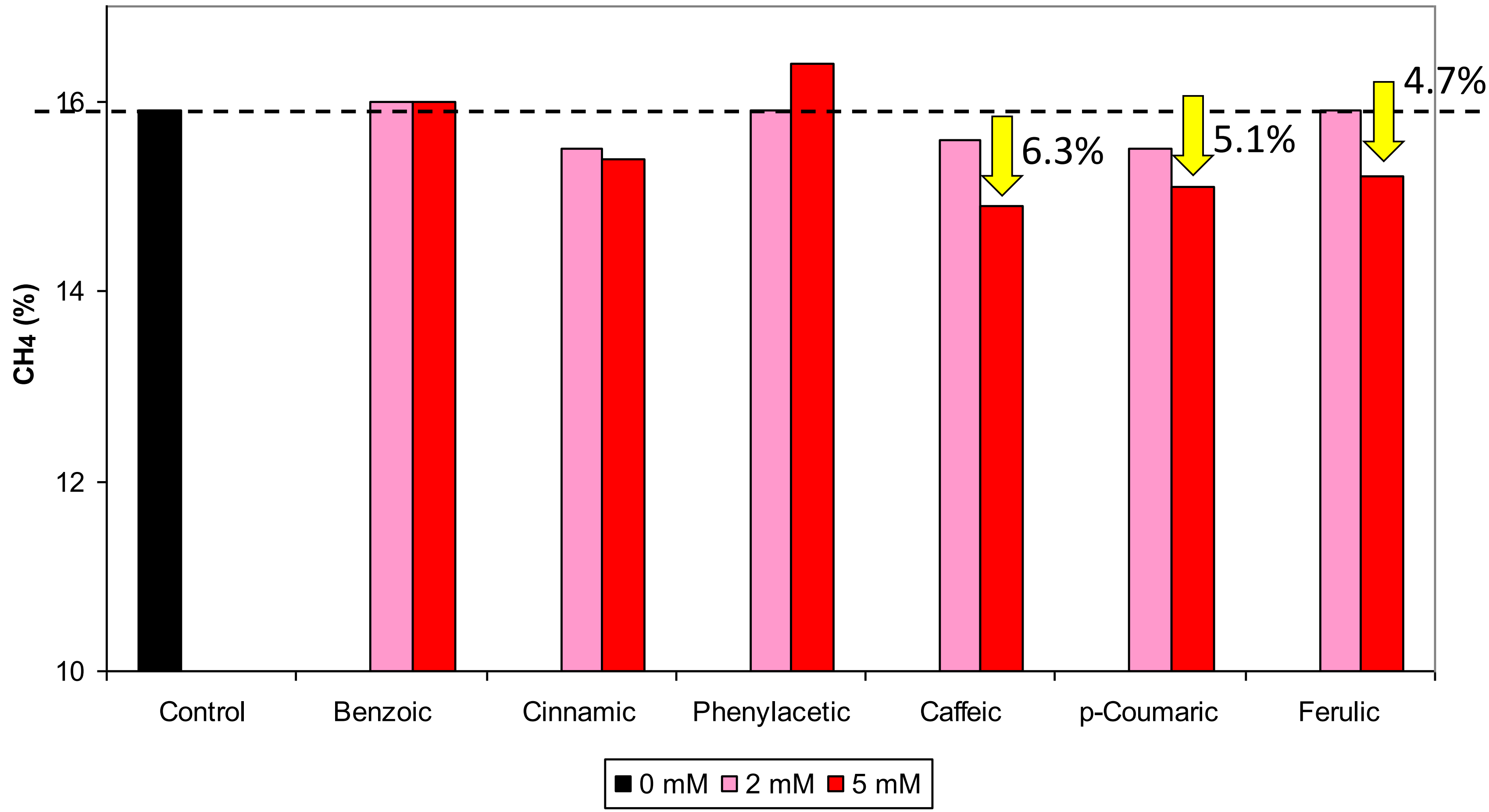


p-coumaric acid



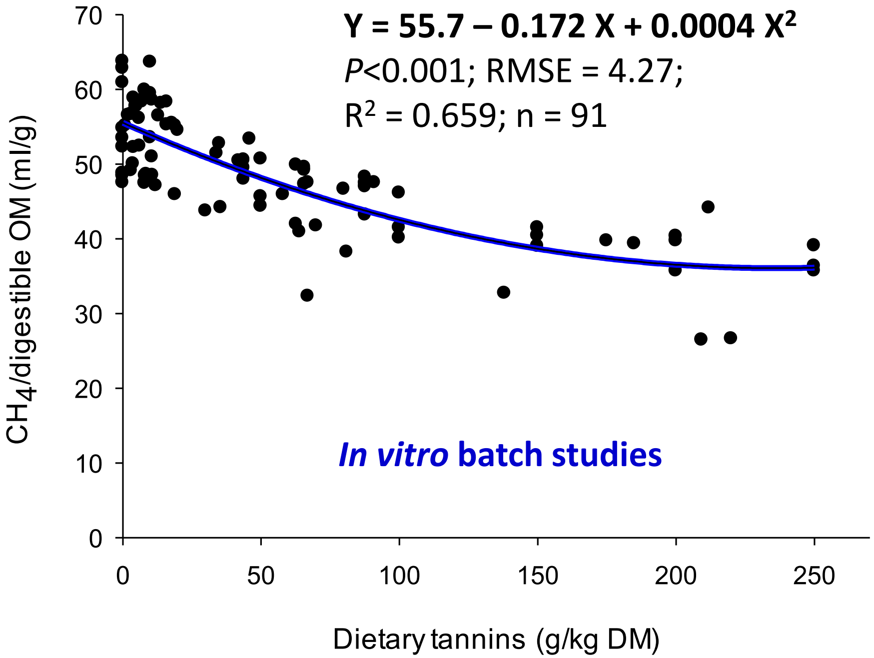
Ferulic acid

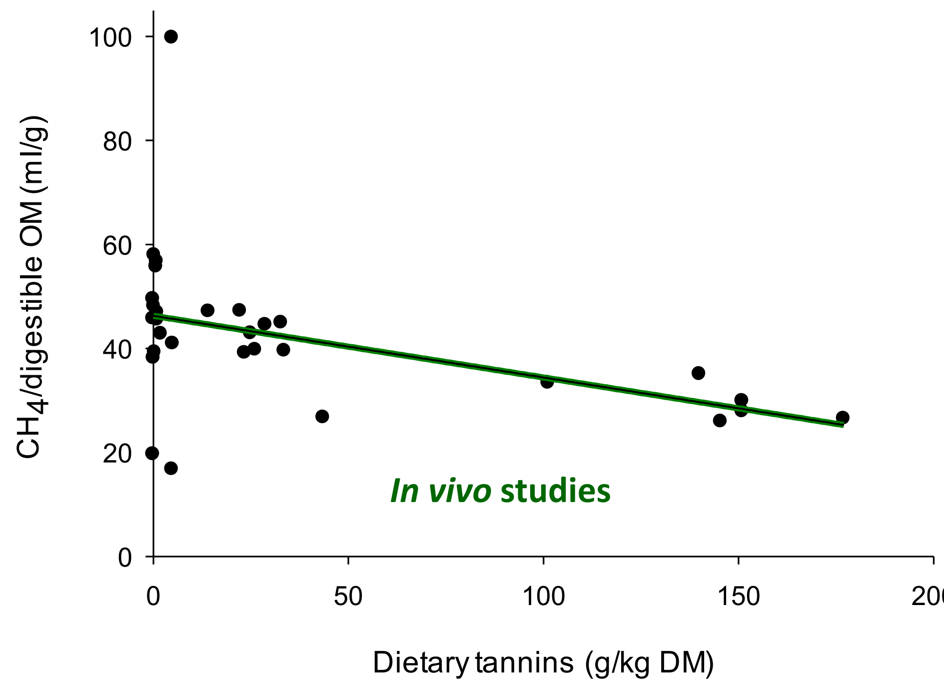
Caffeic, p-coumaric and ferulic acids decreased methane concentration at 5 mM



Methane concentration (ml methane/100 ml gas)

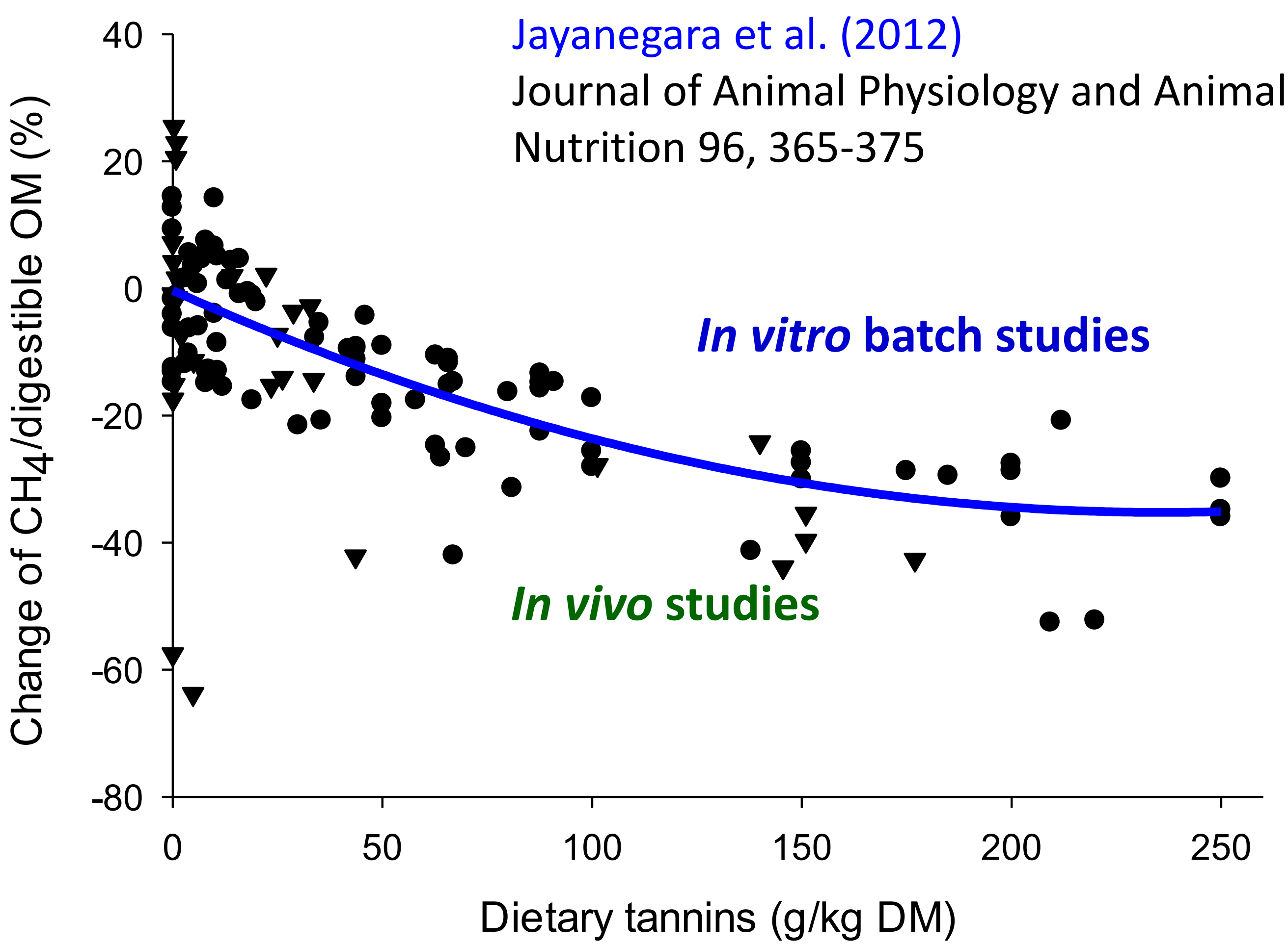
(7) Meta-analysis study





- Database development from literatures
- Total: 30 experiments, 171 treatments
  - *In vitro* batch: 15 experiments, 130 treatments
  - *In vivo*: 15 experiments, 41 treatments
- Statistical analysis with mixed-model methodology
- Weighting data by number of replicates/animals in each experiment

Jayanegara et al. (2012)
Journal of Animal Physiology and Animal Nutrition 96, 365-375

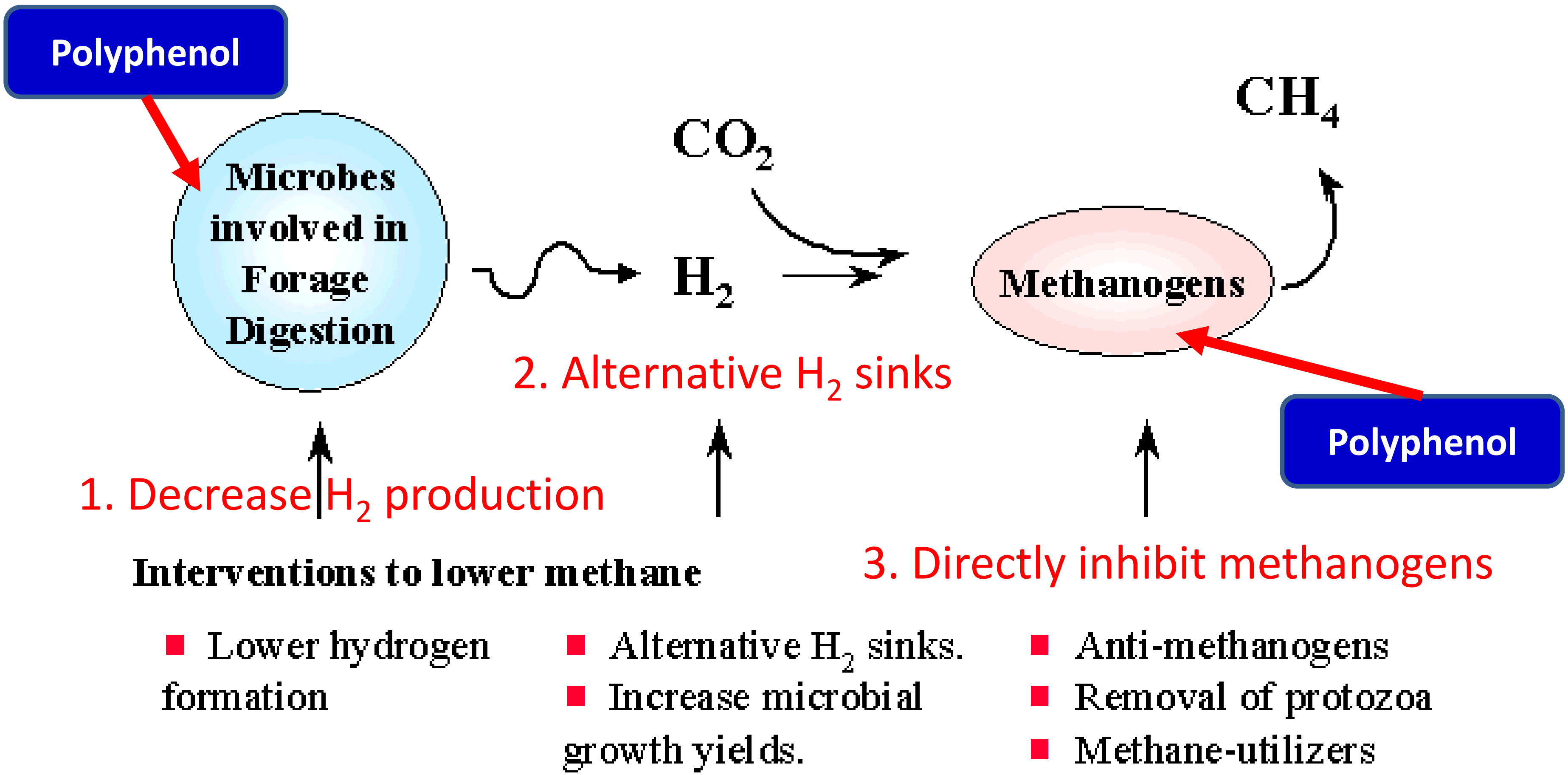


Jayanegara et al. (2012)
Journal of Animal Physiology and Animal Nutrition 96, 365-375
In vitro batch studies
In vivo studies
Change of CH_4/digestible OM (%)
Dietary tannins (g/kg DM)
40
20
0
-20
-40
-60
-80
0
50
100
150
200
250

Principles in mitigating methane

Possible microbial-intervention sites for lowering ruminant methane

Formation of Methane by Microbes in the Rumen

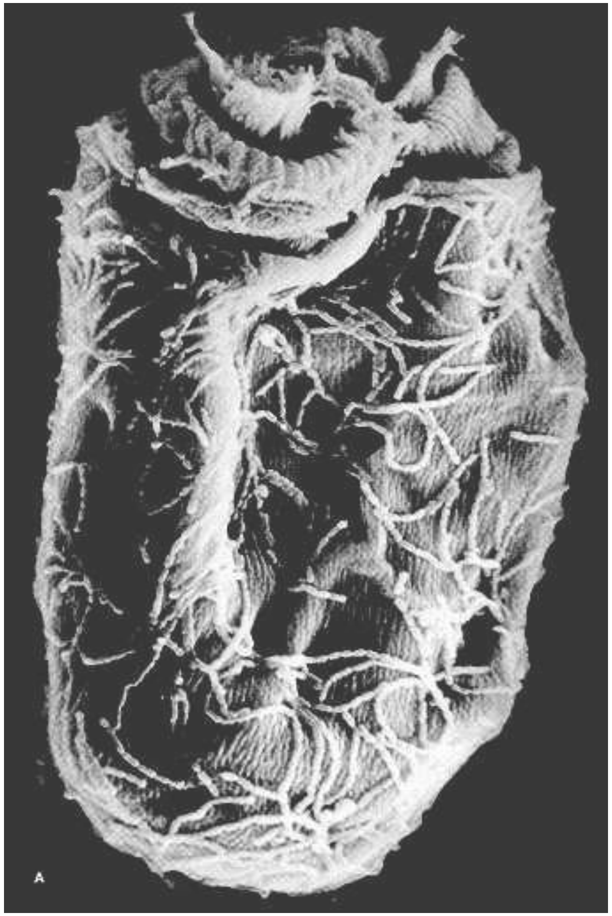


Mode of action

Item	Tannin concentration[1] (% of TMR,[2] DM basis)					
	0	5	10	15	20	25
24-h gas production (mL/200 mg of DM)	43.2[a]	40.8[b]	37.2[c]	34.0[d]	30.8[e]	30.5[e]
CH_4						
% of total gas	18.8	18.2	16.2	17.1	17.2	17.2
mM/g of DM	1.90[a]	1.67[ab]	1.31[bc]	1.19[c]	1.05[c]	1.04[c]
Archaeal population (% of 16S rRNA)	12.0[a]	9.6[b]	8.7[bc]	8.8[bc]	8.8[bc]	7.8[c]
Bacterial population (% of 16S rRNA)	68.4[cd]	69.4[bc]	66.0[d]	72.0[b]	67.8[cd]	78.0[a]
Protozoal population (×10,000/mL)	9.28[a]	8.91[b]	7.81[c]	6.33[c]	6.64[c]	4.14[c]
IVDMD[3]	0.665[a]	0.651[a]	0.650[a]	0.642[b]	0.630[b]	0.625[c]
Fermentation product[4]						
pH	6.32[b]	6.38[ab]	6.32[b]	6.39[ab]	6.43[a]	6.44[a]
NH_3-N (mg/dL)	8.18[a]	6.63[b]	6.13[bc]	5.75[cd]	5.40[d]	5.53[cd]
Total VFA (mM)	66.7[a]	64.1[ab]	61.6[bc]	59.1[cd]	57.1[d]	56.1[d]
C2 (mM)	43.8[a]	42.0[b]	40.6[bc]	39.4[cd]	38.0[de]	37.6[e]
C3 (mM)	12.2[a]	12.0[a]	11.7[a]	11.0[b]	10.4[c]	10.2[c]
C2:C3	3.59[ab]	3.50[b]	3.47[b]	3.58[ab]	3.65[a]	3.68[a]
C4 (mM)	7.48[a]	7.16[a]	6.56[b]	6.36[b]	6.24[b]	6.22[b]
C5 (mM)	1.00[a]	0.83[a]	0.94[a]	0.65[b]	0.78[a]	0.69[b]
iC4+ iC5 (mM)	2.32[a]	2.10[b]	1.81[c]	1.65[d]	1.70[cd]	1.76[cd]
CH_4:total VFA	35.1[a]	38.4[b]	47.0[c]	49.7[d]	54.4[e]	53.9[f]

Source: Bhatta et al. (2009)
J. Dairy Sci. 92, 5512-5522

Tannins and microbial population



Protozoa colonized by methanogens

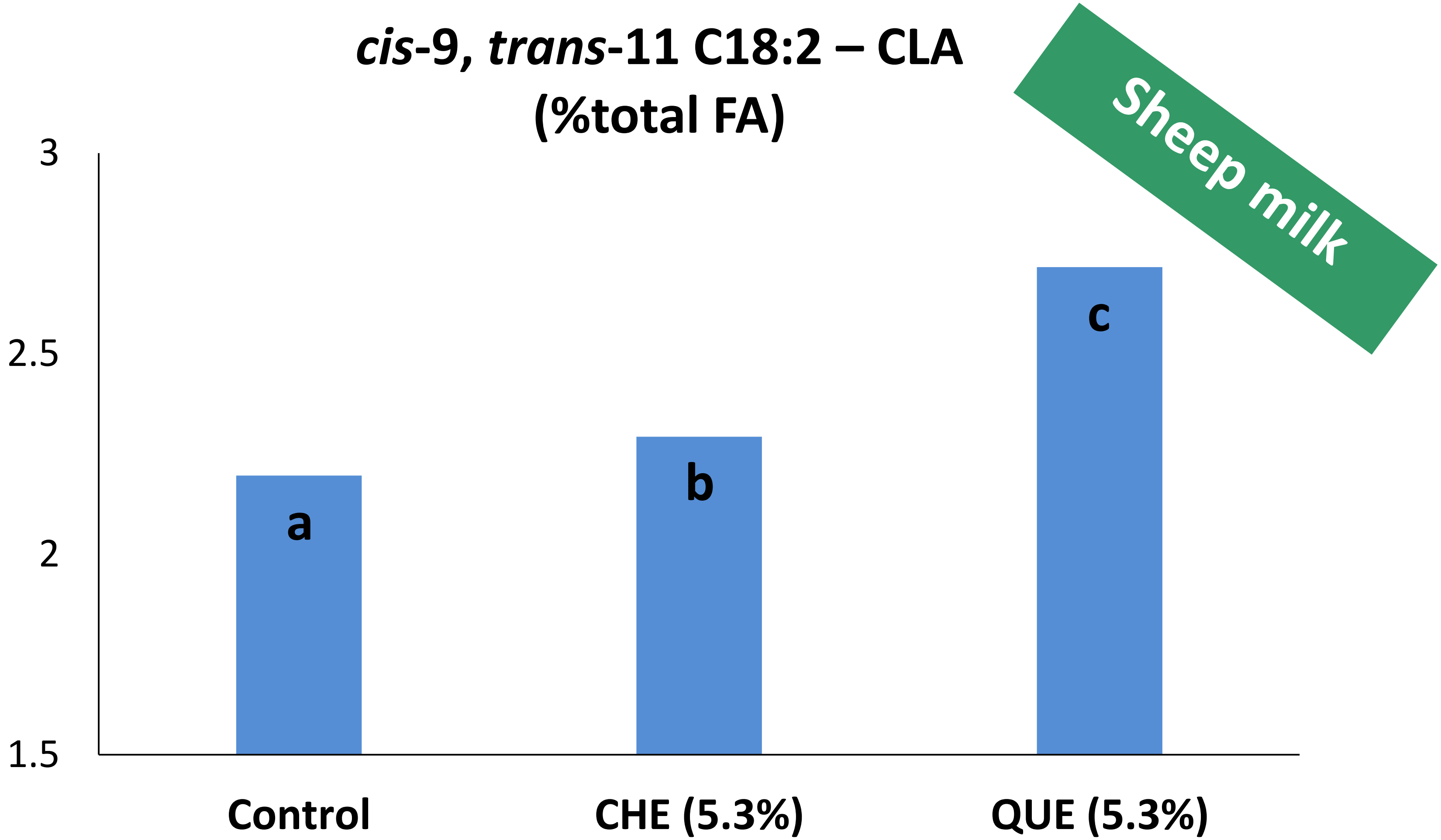
Ruminal methanogens attached to protozoal species → interspecies H transfer

Protozoa-associated methanogens contribute up to 37% of total rumen methane emissions

Removal of protozoa from the rumen (defaunation) → may ↓ CH_4 emission

E. Effects on product quality

Milk FA



Buccioni et al. (2017)
Small Rum. Res. 153, 23-30

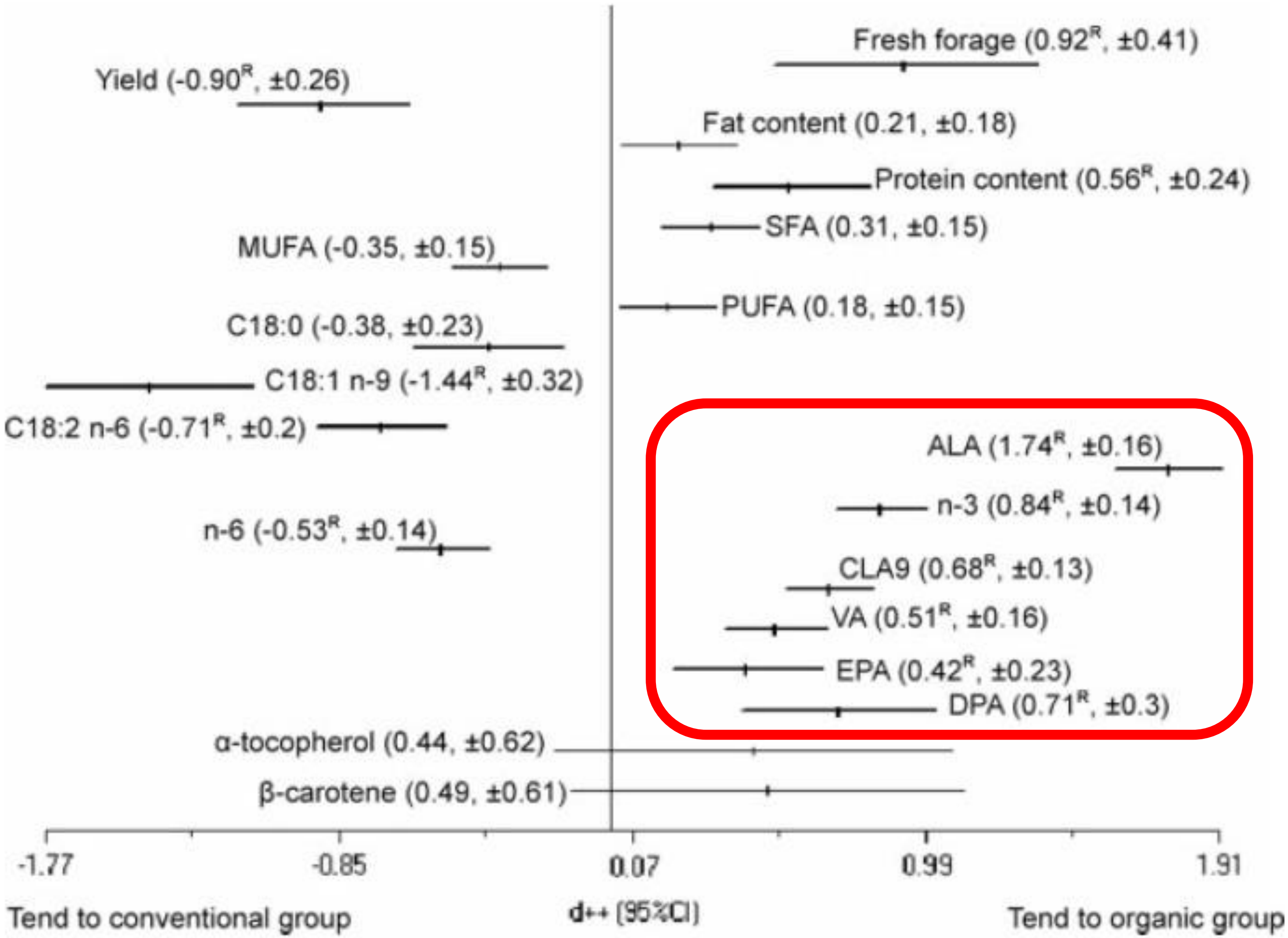
Sheep milk

Fatty acid	Control	TAN3	TAN6	TAN9	Lin	Quad
SFA	62.5	60.6	59.9	59.3	*	ns
PUFA	2.89	3.04	3.33	3.43	*	ns
n-3 PUFA	0.407	0.459	0.511	0.491	*	ns
n-6 PUFA	1.91	1.84	2.15	2.26	*	ns
n-9 MUFA	0.816	0.547	0.598	0.760	ns	**
SFA/PUFA	22.0	20.2	18.9	17.9	*	ns

Abo-Donia et al. (2017)
Livest. Sci. 204, 16-24

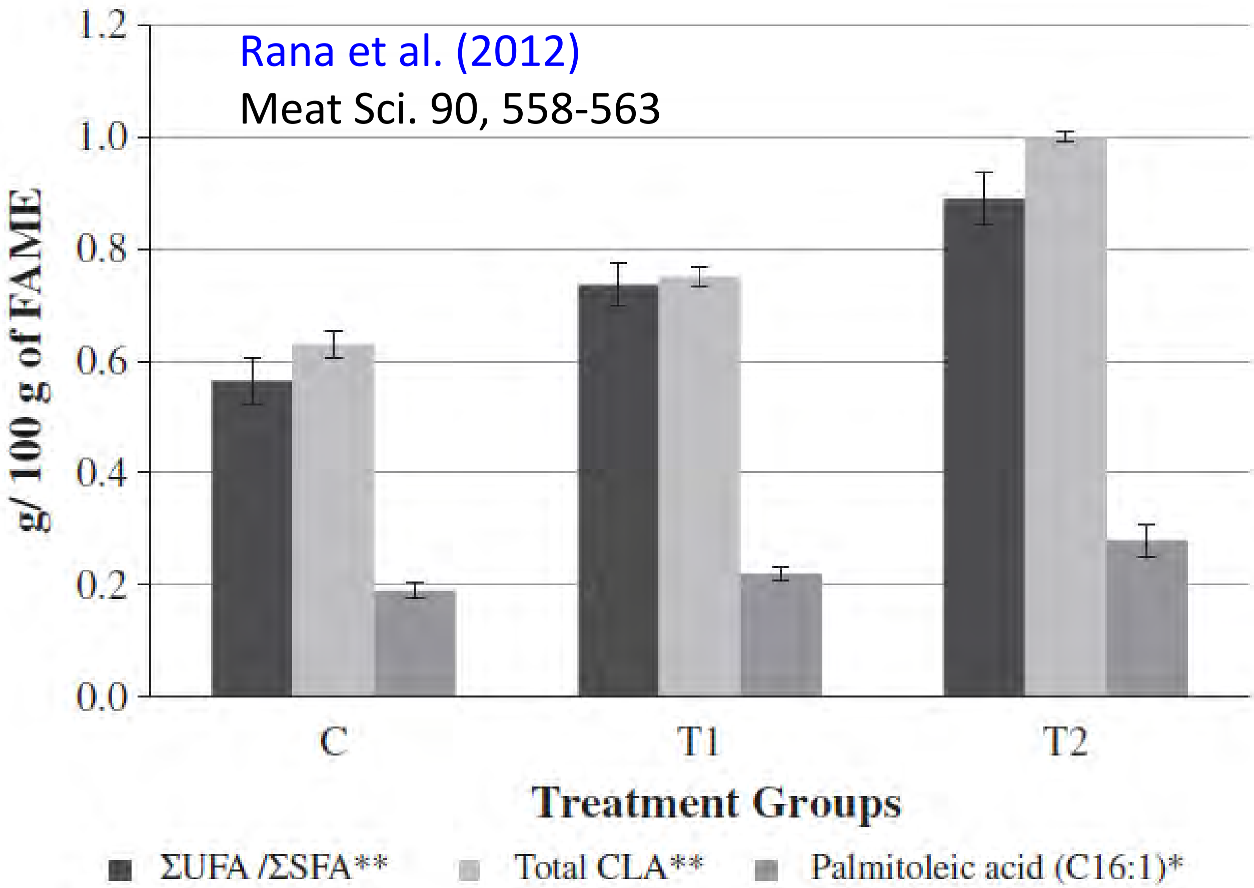
Palupi et al. (2012)
J. Sci. Food Agric. 92, 2774-2781

Cow milk



Rana et al. (2012)

Meat Sci. 90, 558-563



T1: 0.59% DMI

T2: 1.79% DMI

T. chebula extract containing 50% tannin

Graph 1. Effect of *T. chebula* extract supplementation on Δ9-desaturase activity followed with changes in desaturation indices and CLA content in *longissimus dorsi* muscle of kids. Bars indicate SE (standard error). (Significance of effects $^*P<0.05$, $^{**}P<0.01$). ($\sum$UFA/$\sum$SFA or ($\sum$MUFA + $\sum$PUFA/$\sum$SFA): desaturation indices.

Sheep meat

Item	Alfalfa	Red clover	Birdsfoot trefoil	Sainfoin	P-value
CT (%)	0	0	2.1	10.4	
Inmus fat (%)	9.53b	12.5c	6.43a	5.05a	***
n-3 PUFA (%)	5.0a	4.5a	5.6a	9.1b	***
n-6 PUFA (%)	6.3a	4.9a	9.1b	10.9b	***
SFA (%)	50.5b	53.4b	46.8a	44.0a	***
PUFA (%)	14.2a	11.9a	17.9b	23.4c	***
PUFA/SFA	0.28a	0.22a	0.38b	0.53c	***

Girard et al. (2016)
J. Sci. Food Agric. 96, 1923-1933

Sheep meat

Item	Control	QT 2%	QT 4%	P-value
C18:0	19.8b	16.3a	16.7a	***
C18:1 *trans*-9	1.82a	2.17b	2.52b	*
CLA *cis*- *trans*-11	0.40a	0.46a	0.59b	***
Total CLA	0.55a	0.67ab	0.78b	***
SFA	50.8b	48.4a	46.9a	***
UFA	49.2a	51.6a	53.1b	***
PUFA	7.41a	8.54a	10.5b	***

Kamel et al. (2018)
Anim. Feed Sci. Technol. 235, 97-104

Mode of action

Inhibition of phenolics on lipase activity

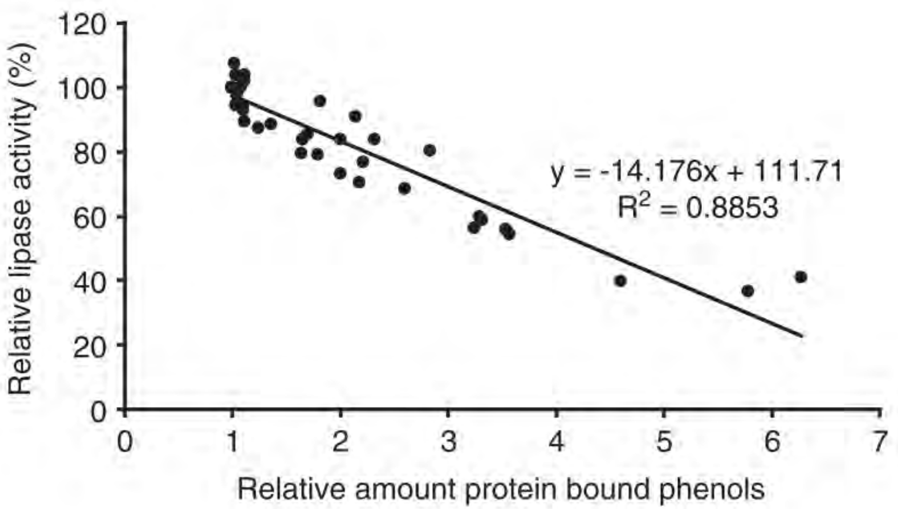


Figure 1 Relation between relative amount of bound phenols and relative lipase activity in a protein extract of red clover after incubation (120 s) with 4-methyl catechol, both were calculated as the amount or activity divided by the amount or activity present in the corresponding fresh forage (adapted from data presented in Van Ranst *et al.*, 2009b).

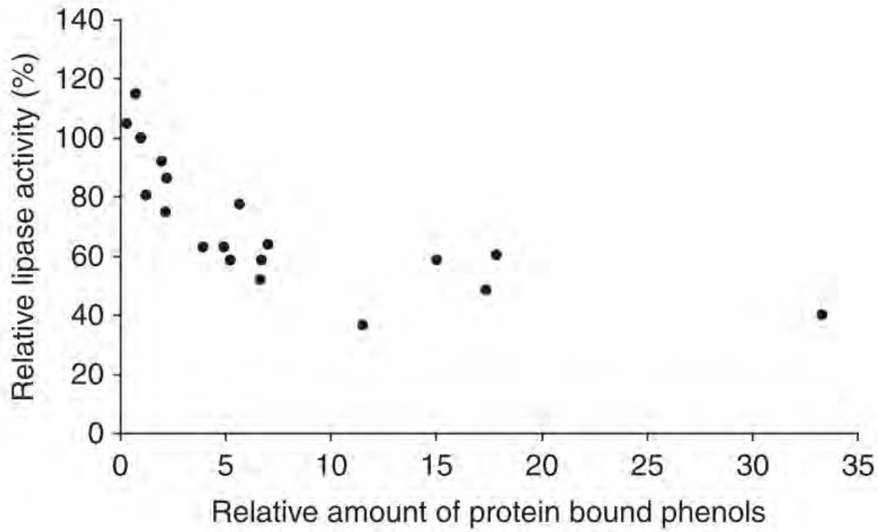
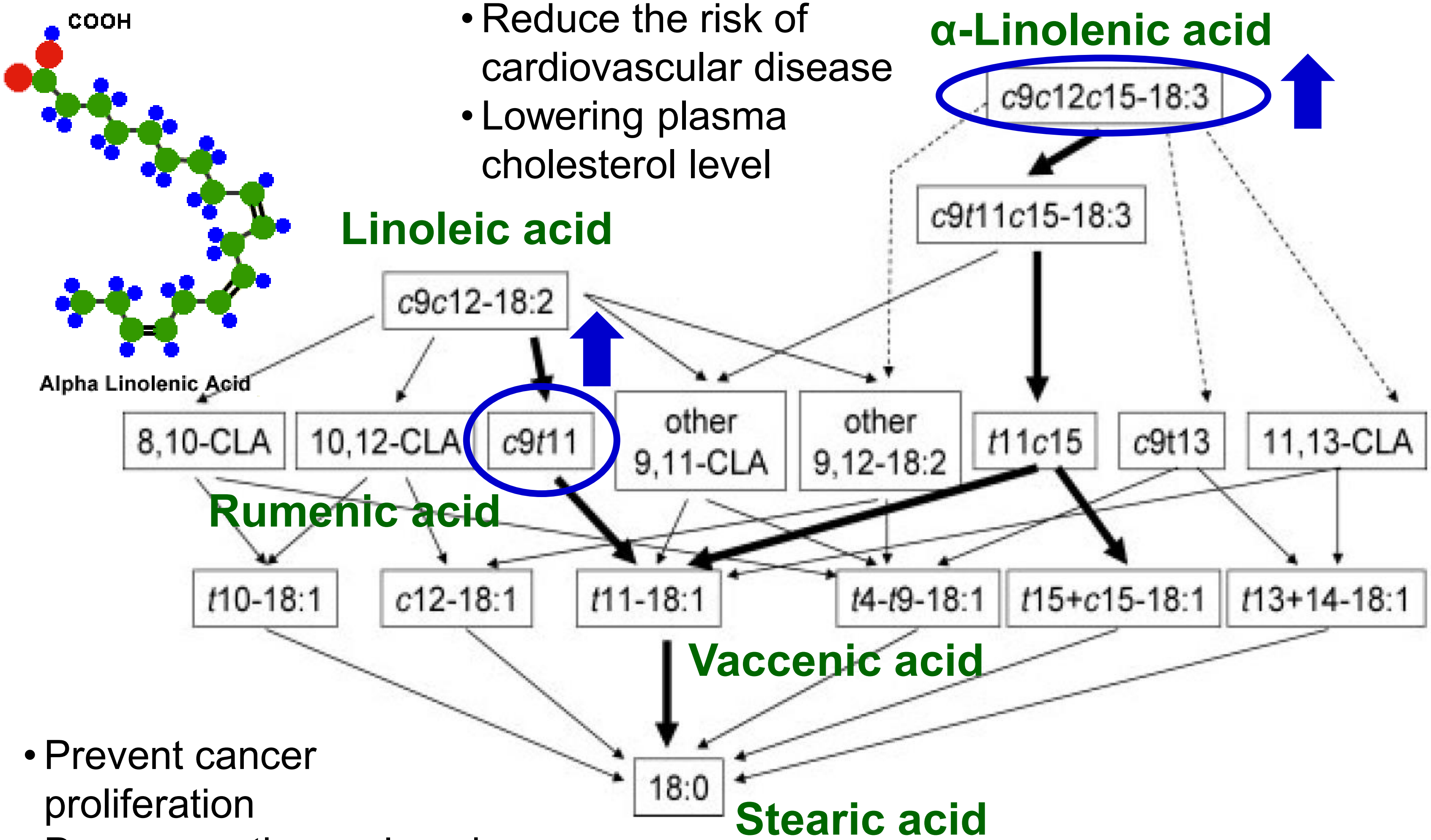


Figure 2 Relation between relative amount of bound phenols and relative lipase activity in a protein extract of undamaged, crushed or frozen/thawed red clover which was wilted for 4 or 24 h, both were calculated as the amount or activity divided by the amount or activity present in the corresponding fresh forage (adapted from data presented in Van Ranst *et al.*, 2009b).

Source: Van Ranst et al. (2011)
Animal 5, 512-521

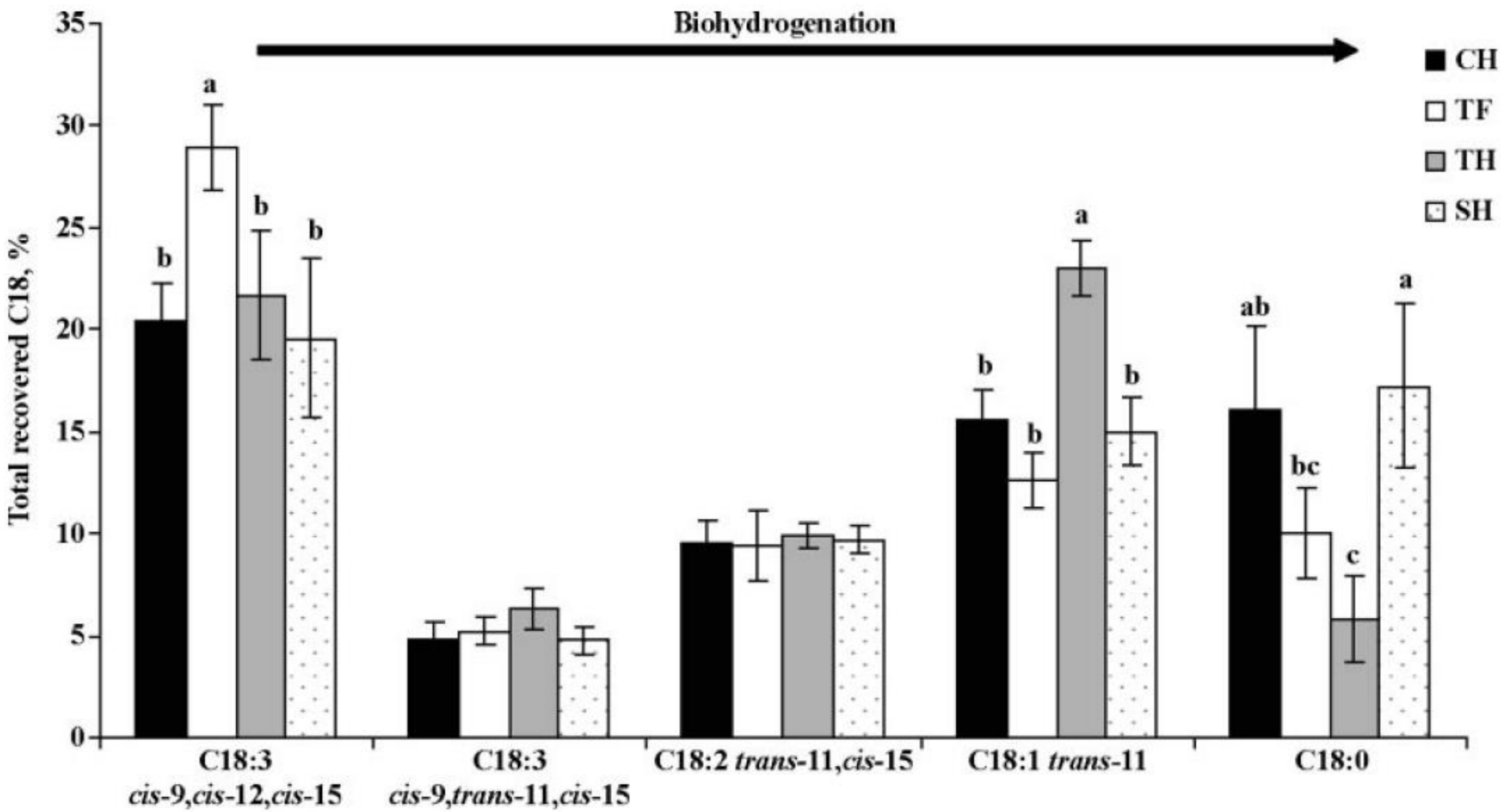
Modulation of PUFA biohydrogenation

- Reduce the risk of cardiovascular disease
- Lowering plasma cholesterol level



- Prevent cancer proliferation
- Decrease atherosclerosis
- Improve immune system

Chilliard et al. (2007)
Eur. J. Lipid Sci. Technol. 109, 828-855



Condensed tannins (CT)

Khiaosa-ard et al. (2009)

J. Dairy Sci. 92, 177-188

CH: grass-clover hay (control)

TF: dried sainfoin (7.9% CT)

TH: CH + *A. mearnsii* extract (7.9% CT)

SH: CH + *Y. schidigera* extract (1.1% saponins)

Incubation of tropical plants *in vitro*

Correlation coefficients between plant chemical composition and disappearance and appearance of C18 fatty acids (n = 27)

Fatty acid	Total phenols	Non-tannin phenols	Total tannins	Condensed tannins	Hydrolysable tannins
Disappearance					
C18:3 *n*-3	–0.69***	–0.25	–0.65***	–0.24	–0.63***
C18:2 *n*-6	–0.72***	–0.23	–0.69***	–0.34	–0.64***
C18:1 *n*-9	–0.76***	–0.42*	–0.66***	–0.73***	–0.46*
Appearance					
*c*9,*t*11-C18:2	0.55**	0.21	0.52**	0.64***	0.33
*t*11-C18:1	–0.01	0.02	–0.02	0.26	–0.12
C18:0	–0.47*	–0.22	–0.43*	–0.44*	–0.31

*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001

Jayanegara et al. (2011)
Anim. Prod. Sci. 51, 1127-1136

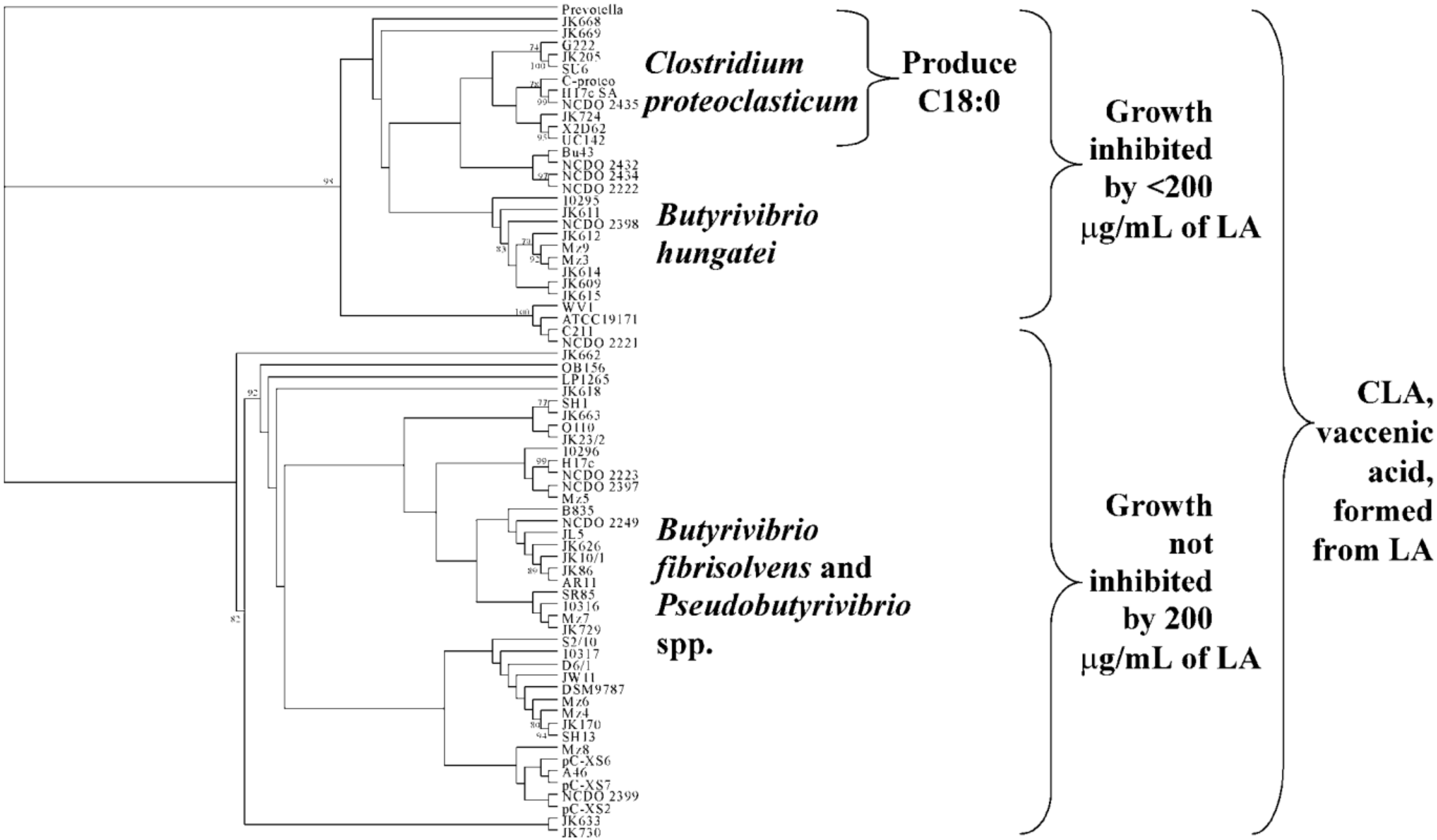
Incubation of alpine plants *in vitro*

Correlation coefficients between plant chemical composition and disappearance and appearance of C18 fatty acids (n = 18)

Fatty acid	Total phenols	Non-tannin phenols	Total tannins	Condensed tannins	Hydrolysable tannins
Disappearance					
C18:3 *n*-3	–0.72**	0.07	–0.84***	0.02	–0.88***
C18:2 *n*-6	–0.70**	0.03	–0.80***	0.03	–0.83***
C18:1 *n*-9	0.37	0.02	0.41	–0.01	0.43
Appearance					
*c*9,*t*11-C18:2	0.46	0.18	0.46	0.09	0.47*
*t*11-C18:1	0.81***	0.30	0.81***	0.42	0.79***
C18:0	–0.57*	0.14	–0.69**	0.07	–0.73**

*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001

Jayanegara et al. (2012)
Livest. Sci. 147, 104-112



Microbes involved in fatty acid biohydrogenation

Jenkins et al. (2008)
J. Anim. Sci. 86, 397-412

Influence of tannins on biohydrogenating bacteria

TABLE 3. Effect of tannin supplementation on rumen content microbial population and on LA-I activity

Ruminal ecosystem parameter	Value for indicated dietary treatment: Concentrate (n = 7)[a]	Value for indicated dietary treatment: Concentrate plus tannins (n = 6)[a]	SEM	Significance
Microbial population				
No. of protozoa ($\log_{10}$ copies/g FM[c])	5.01	7.82	0.506	0.001
No. of bacteria ($\log_{10}$ copies/g of FM)	9.55	9.64	0.134	0.766
B. fibrisolvens (% of total bacteria)	4.22	8.76	1.044	0.022
B. proteoclasticus (% total bacteria)	3.99	2.77	0.363	0.094
Amount of microbial protein (mg/ml)	6.33	16.23	1.5	<0.0005
CLA production (nmol/ml/min)[b]	2.47×10^5	2.13×10^5	3.27×10^4	0.693

[a] n, number of sheep.
[b] LA-I assay.
[c] FM, fresh matter.

Containing 6.4% tannins from quebracho powder

Vasta et al. (2010)
Appl. Environ. Microbiol. 76, 2549-2555

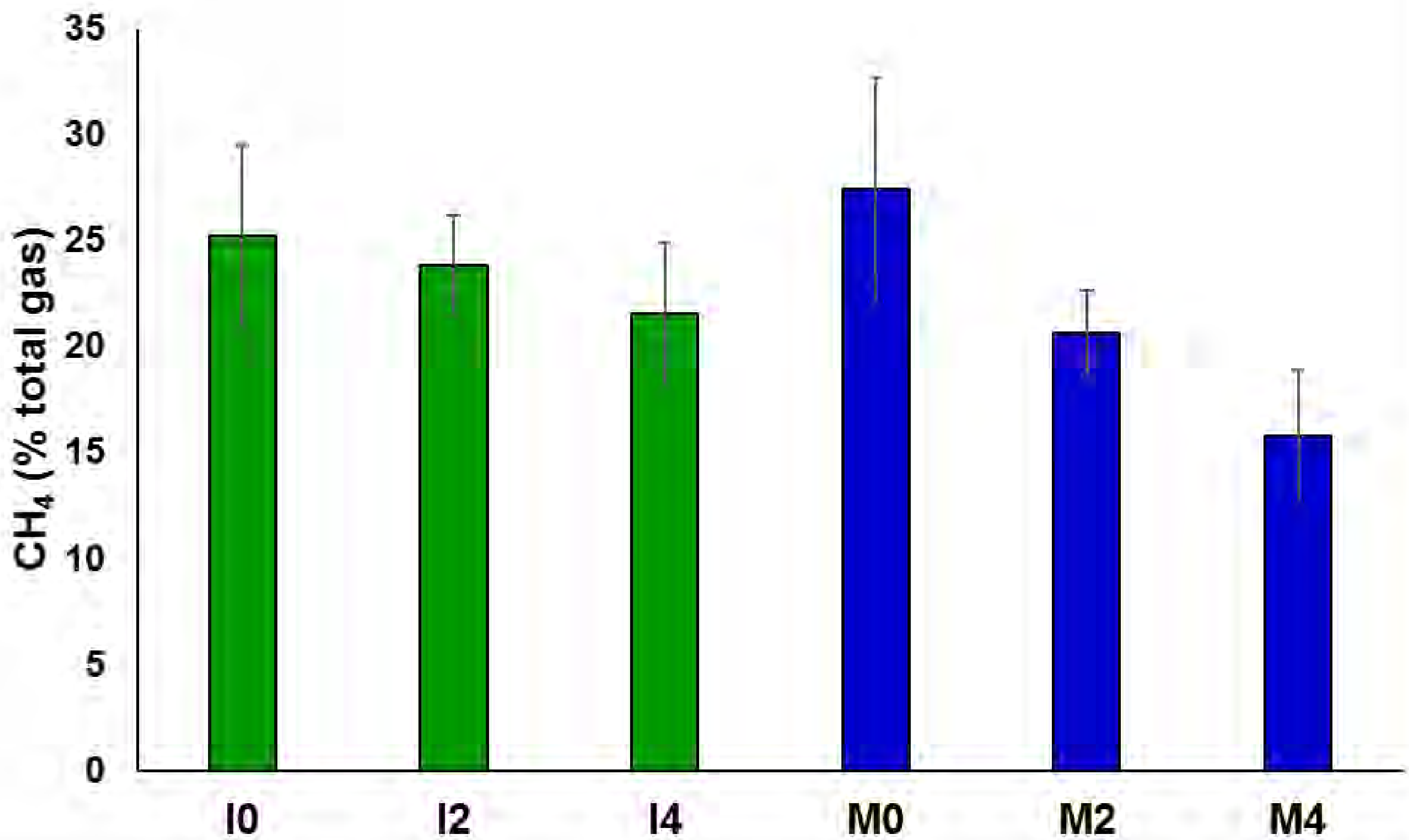
F. An on going study: polyphenols as silage additives

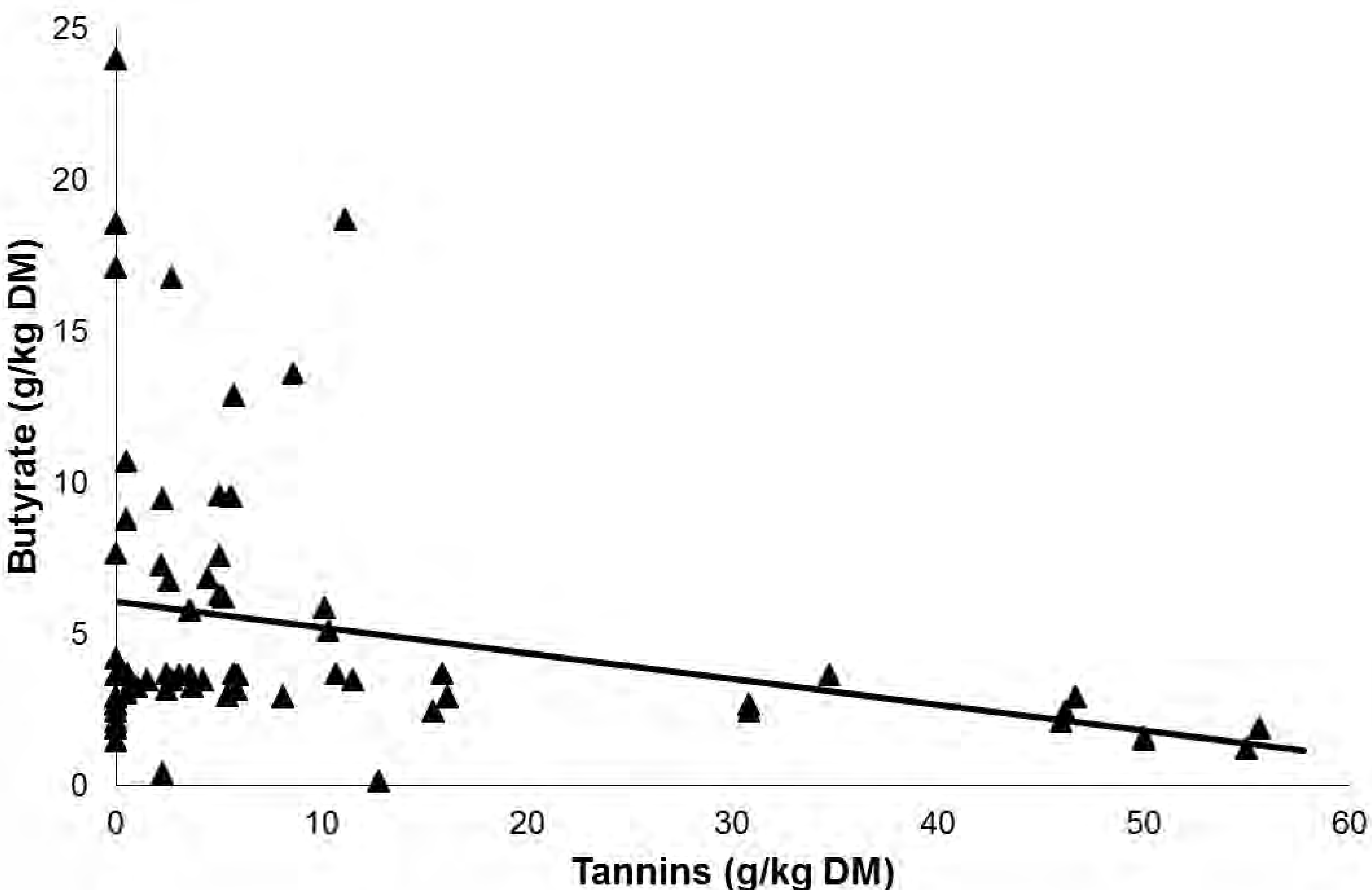
Silage fermentation

Jayanegara et al. (2018)
Vet. World, in preparation

Forage	Tannin (%DM)	DM (%)	DM loss (%)	pH	VFA (mmol/l)	NH_3 (mmol/l)
Indigofera	0	20.1	1.56	5.37^{c}	153^{d}	13.0^{c}
	2	20.1	2.07	5.27^{bc}	143^{c}	10.5^{bc}
	4	20.7	2.04	5.57^{c}	138^{bc}	8.4^{ab}
Moringa	0	20.1	1.49	5.00^{ab}	138^{bc}	10.9^{bc}
	2	20.1	4.55	4.97^{ab}	129^{b}	8.4^{ab}
	4	20.5	2.83	4.87^{a}	116^{a}	6.0^{a}
SEM		0.254	0.368	0.070	3.12	0.610
P-value						
Forage		0.941	0.119	<0.001	<0.001	0.006
Tannin		0.692	0.112	0.647	<0.001	<0.001
Forage× Tannin		0.989	0.287	0.178	0.545	0.976

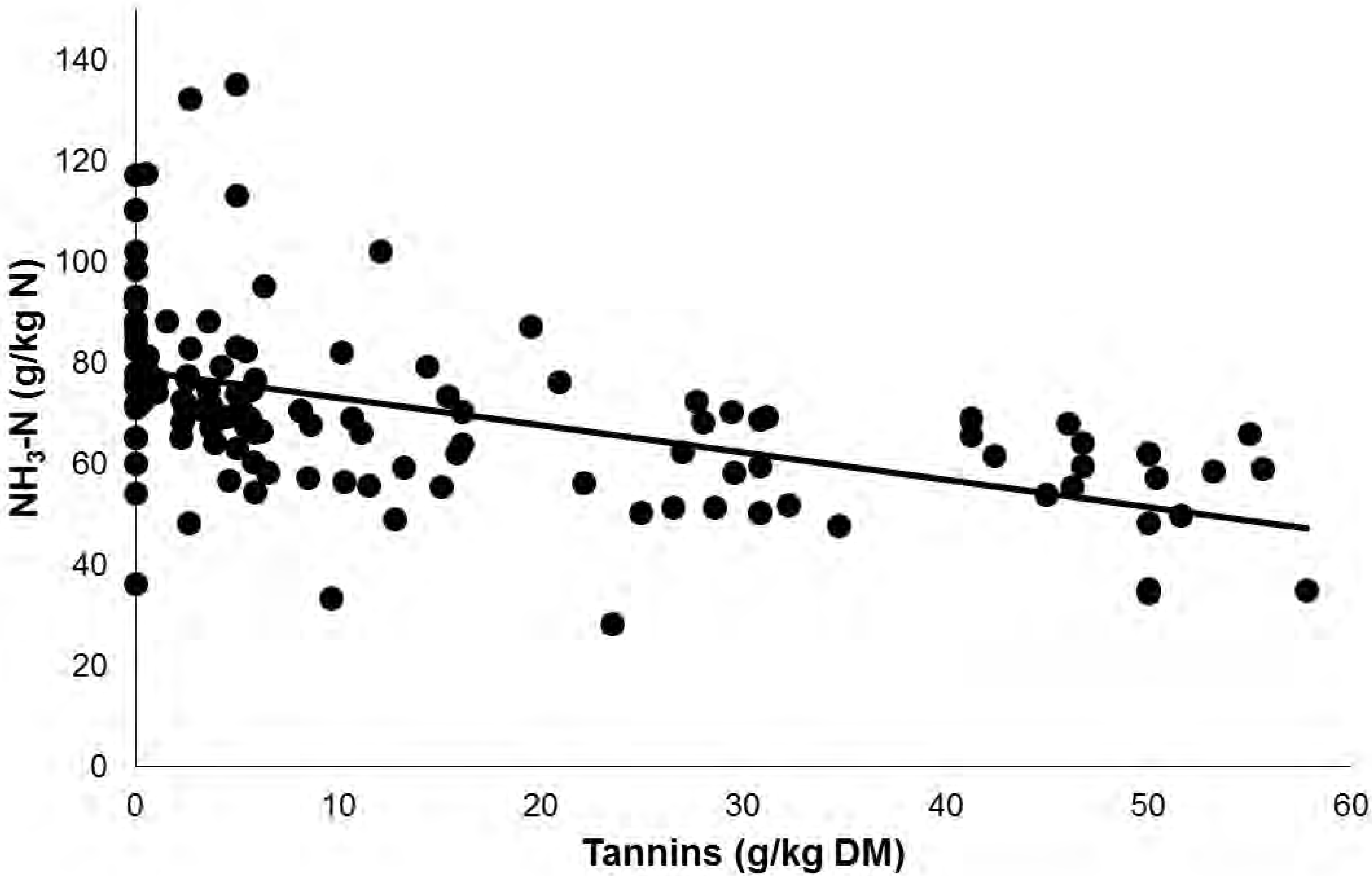
Jayanegara et al. (2018)
Vet. World, in preparation





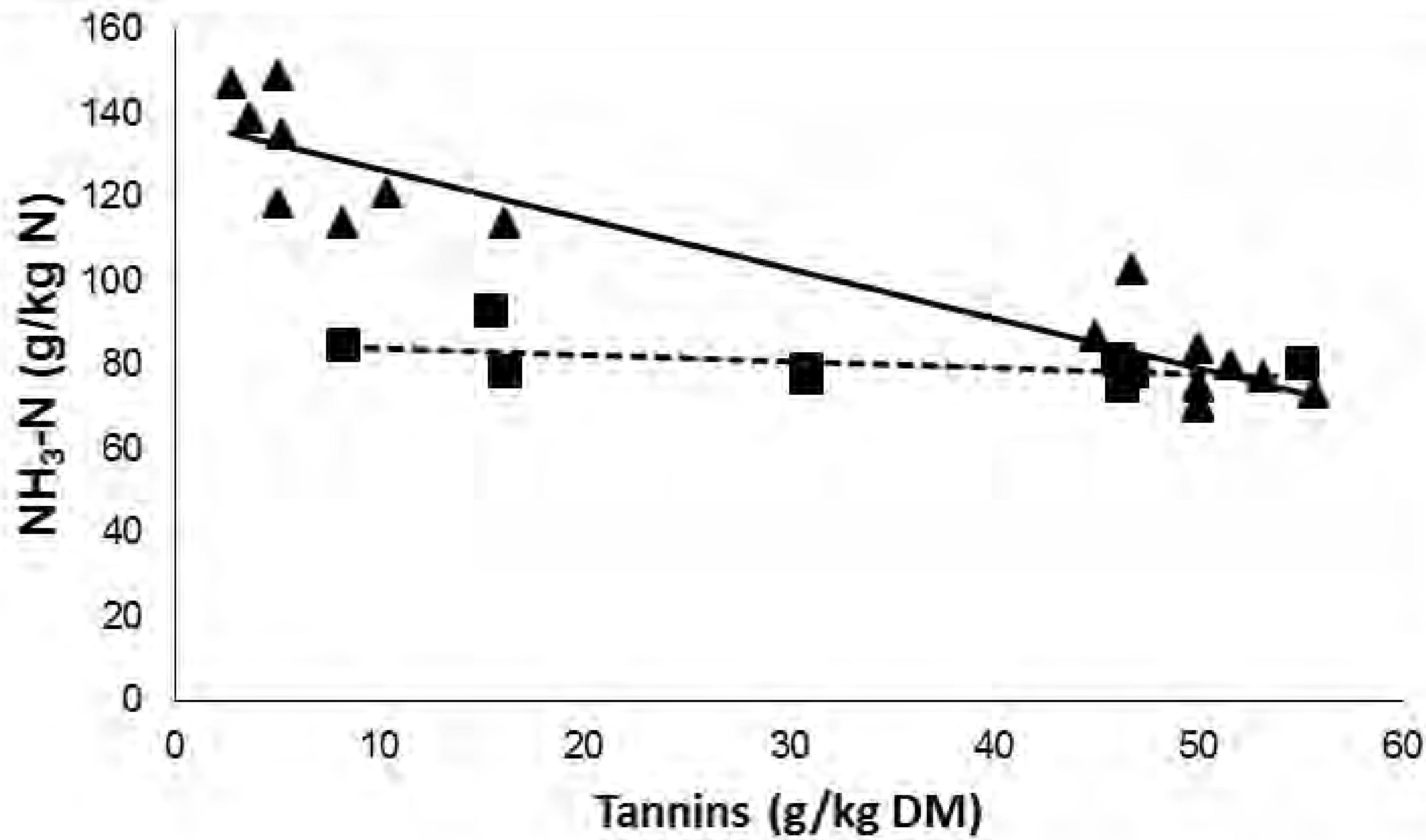
Jayanegara et al. (2018)

Anim. Feed Sci. Technol., in revision



Jayanegara et al. (2018)

Anim. Feed Sci. Technol., in revision



Jayanegara et al. (2018)

Anim. Feed Sci. Technol., in revision

ACKNOWLEDGMENT

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Thank you for your attention!