Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/j.1439-0396.2011.01172.x

REVIEW ARTICLE

Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments

A. Jayanegara, F. Leiber and M. Kreuzer

ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland

Keywords
ruminant, rumen, tannin, methane, meta-analysis

Correspondence
M. Kreuzer, ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland. Tel: +41 44 632 5972; Fax: +41 44 632 1128; E-mail: mkreuzer@ethz.ch

Received: 14 January 2011;
accepted: 22 April 2011

Summary

A meta-analysis was conducted to evaluate the extent to which dietary tannin level is related to methane emissions from ruminants. Data from a total of 30 experiments comprising 171 treatments were entered in a database. *In vitro* batch culture and *in vivo* measurements were distinguished as experimental approaches. With any approach, methane declined when dietary tannins increased. The *in vitro* approach predicted the *in vivo* response quite accurately. However, *in vitro*, the response followed a quadratic response pattern (R^2 = 0.66; lower response with increasing tannin level), whereas *in vivo*, this decline was linear (R^2 = 0.29). This indicates that the *in vitro* batch culture is of limited accuracy for estimating effects at levels >100 g tannin/kg dry matter. The large variation in methane/digestible organic matter (OM) found at low tannin levels may explain contrasting literature reports. Methane reduction with tannins was associated with a reduced apparent digestion of OM, and especially fibre, but methane/apparently digestible OM declined also. The present findings are helpful as they identified an underlying general antimethanogenic effect of tannins across tannin sources and experimental conditions, thus allowing concentrating the search on sources with satisfactory palatability and low adverse effects on animal performance.

Introduction

Tannins represent an important class of plant secondary metabolites and are produced by the plants in their intermediary metabolism. Chemically, they are polyphenolic compounds with varying molecular weights, and they have the ability to bind natural polymers such as proteins and carbohydrates (Mueller-Harvey, 2006). Based on their molecular structure, tannins are classified as either hydrolysable tannins (HT; polyesters of gallic acid and various individual sugars) or condensed tannins (CT; polymers of flavonoids), although there are also tannins that represent combinations of these two basic structures (McSweeney et al., 2001). With respect to ruminant nutrition, tannins are considered to have both beneficial and detrimental nutritional effects (Reed, 1995). The beneficial effects may include better utilization of dietary protein, faster body weight or wool growth, higher milk yield, increased fertility, and improved animal welfare and health through the prevention of bloat and reduced worm burdens (Mueller-Harvey, 2006). However, the expression of beneficial effects depends on the source and level of tannins applied. Negative effects of tannins have been associated with their potential toxicity to some

rumen micro-organisms (Goel et al., 2005) and in the metabolism of the ruminant, in particular when HT are involved (Reed, 1995). Other adverse effects include low palatability and impaired diet digestibility, resulting in a reduced performance (Makkar, 2003; Tiemann et al., 2008a).

Research on mitigating methane (CH_4) emissions from ruminants has received a great attention especially during the last decade. It was estimated that, globally, ruminants produce 80 million tonnes of CH_4 annually, which accounts for 28% of anthropogenic emissions (Beauchemin et al., 2008). Tannins have been considered to represent a promising group of compounds for decreasing enteric CH_4 emissions from ruminants by dietary means (Carulla et al., 2005; Animut et al., 2008a,b; Bhatta et al., 2009). However, it seems that findings on whether tannins are able to genuinely suppress ruminal CH_4 formation per unit of digestible nutrient, and the extent to which this effect occurs, appear to be inconsistent. For example, Beauchemin et al. (2007) found that feeding up to 20 g quebracho (*Schinopsis* sp.) tannin extract (a source of CT) per kg of dietary dry matter (DM) failed to reduce enteric CH_4 emissions from growing cattle, although the protein-binding effect of the CT was evident. Further publications reported a lack of effect on CH_4 emissions of chestnut (*Castanea sativa*) wood tannins (Śliwiński et al., 2002a,b) and sorghum (*Sorghum bicolor*) tannins (De Oliveira et al., 2007). It remains unclear whether these sources of CT were simply inefficient or whether the level of supplementation was too low.

The objectives addressed in the present review and evaluation of the literature were (i) to clarify whether there is a general underlying efficiency of tannins against CH_4 formation in the rumen, (ii) to describe the associated tannin effects on digestibility and rumen fermentation and (iii) to compare results obtained with *in vitro* and *in vivo* methods. Meta-analysis was applied as a statistical tool to deal with these items.

Material and methods

Database development

A database was constructed from experiments where both dietary tannin concentrations and CH_4 emission were specified. This included publications and own unpublished experiments. Publications were obtained from the ISI Web of Science database using phenol, tannin, methane and/or rumen as keywords. In addition to CH_4 emission, related variables were also integrated in the database. These variables included *in vitro* and *in vivo* digestibility, body N retention, as well as ruminal pH, gas production, short-chain fatty acid (SCFA) profiles, ammonia concentration and bacterial and protozoal counts. Tannins applied in the studies were originating from plants where tannins either remained in the original matrix or had been extracted. The tannin form was HT, CT, unspecified or represented a mixture of different tannins forms. In the meta-analysis, form of tannins was not distinguished by statement as a categorical variable as there were very few studies that reported the effects of HT, particularly *in vivo*. Treatments where polyethylene glycol (PEG) had been added were excluded from the database because the purpose of adding this compound is to neutralize the effects of tannins under the conditions of the ruminal environment (Makkar, 2003; Tiemann et al., 2008b).

A total of 30 experiments comprising 171 dietary treatments as described in Table 1 (*in vitro* experiments) and Table 2 (*in vivo* experiments) were fed into the database. Experiments were treated individually even when published within one article. The database was segregated into two categories based on different methods or systems applied in the experiments. These were *in vitro* batch culture (15 experiments/130 treatments) and *in vivo* experiments (15/41). Data available from experiments using the *in vitro* continuous culture approaches (rumen simulation technique; Czerkawski and Breckenridge, 1977) were too few (6 experiments/25 treatments, i.e. Śliwiński et al., 2002a; Hess et al., 2006, 2008; Tiemann et al., 2008c; Bekele et al., 2009; Khiaosa-ard et al., 2009) to allow regression analysis of sufficient statistical power. As indicated in Table 1, the *in vitro* batch culture experiments had been conducted using the Hohenheim gas test, the syringe gas generator, glass bottle incubation or batch culture incubation. *In vivo* data included results from cattle, sheep, goats and alpaca. Measurements of CH_4 in the *in vitro* batch experiments had been taken using gas chromatographs, calorimetric system or infrared CH_4 analysers (Table 1), while the measurements in the *in vivo* experiments were taken using respiration calorimetry systems equipped with infrared CH_4 detectors, sulphur hexafluoride (SF_6) tracer technique or gas chromatography after gas sampling (Table 2). In most of the *in vivo* experiments, the animals were fed *ad libitum*, sometimes by grazing pastures of sufficient size. Digestibility data had been never corrected by the amounts of microbial mass attached to the feed

Table 1 *In vitro* experiments (Expt) included in the meta-analysis of the effect of dietary tannin levels on ruminal methane emissions

Expt no.	Reference	*In vitro* method	Basal feed	Tannin source*	Tannin form	Tannin level (g/kg DM)	Gas sampling† (h)	CH_4 method
1	Hayler et al. (1998)	HGT	–	*Phyllantus discoideus*, *Mangifera indica*, *Agelaea obliqua*, *Leucaena leucocephala*	Non-extracted	5.4–69.4	24	GC
2	Min et al. (2005)	SGG	Wheat forage	*Schinopsis* sp. (bark; CT)	Crude extract	0–20‡	12	CS
3	Tavendale et al. (2005)	GBI	–	*Medicago sativa*, *Lotus pedunculatus* (CT)	Non-extracted	0.2 and 99	12	GC
4	Wang et al. (2008)	BCI	Barley silage, alfalfa hay, grass hay	*Ascophyllum nodosum* (PT)	Extract	0 and 40	24	GC
5	Bhatta et al. (2009)	HGT	–	*Terminalia* sp. (HT), *Castanea* sp. (HT), *Schinopsis* sp. (CT), *Acacia* sp. (CT)	Extract	52.7–185‡	24	GC
6	Bhatta et al. (2009)	HGT	Timothy hay, concentrate	*Castanea* sp. (HT), *Schinopsis* sp. (CT)	Extract	0–250‡	24	GC
7	Bhatta et al. (2009)	HGT	Timothy hay, concentrate	*Castanea* sp. (HT), *Schinopsis* sp. (CT)	Extract	0–250‡	24	GC
8	Bhatta et al. (2009)	HGT	Timothy hay, concentrate	*Castanea* sp. (HT), *Acacia* sp. (CT)	Extract	0–250‡	24	GC
9	Jayanegara et al. (2009)	HGT	–	Various Mongolian plants	Non-extracted	4–209.3	24	IR
10	Hariadi and Santoso (2010)	HGT	Elephant grass	Various tropical plants	Non-extracted	0–10.8	48	GC
11	Jayanegara et al. (2011a)	HGT	–	Various tropical plants	Non-extracted	2–220	24	GC
12	Jayanegara et al. (2011b)	HGT	–	Various alpine plants	Non-extracted	0–79	24	GC
13	Own unpublished data	HGT	Grass hay and barley straw	*Rhus typhina*, *Salix alba*	Non-extracted	0–209.3	24	IR
14	Own unpublished data	HGT	Grass hay and concentrate	*Castanea* sp. (HT), *Rhus typhina* (HT), *Acacia* sp. (CT), *Schinopsis* sp. (CT)	Purified extract	0–87.7	24	IR
15	Own unpublished data	HGT	–	Mixed alpine meadows	Non-extracted	2.2–9.2	24	GC

BCI, batch culture incubation; CS, calorimetric system; CT, condensed tannins; GBI, glass bottle incubation; GC, gas chromatograph; HGT, Hohenheim gas test; HT, hydrolysable tannins; IR, infrared; PT, phlorotannins; SGG, syringe gas generator; DM, dry matter.

*Unless tannin source is specified in brackets, the statement refers to mixed tannins.

†Time of incubation when the gas was sampled for CH_4 measurement.

‡Tannin level was not directly measured, but the information was obtained from a supplier.

particles; thus, they represent apparent digestibility only.

Statistical analysis

The analysis of the data assembled in the database was conducted by a statistical meta-analysis approach (St-Pierre, 2001; Sauvant et al., 2008). Using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., 2008), the following model was applied:

$$Y_{ij} = B_0 + B_1 X_{ij} + s_i + b_i X_{ij} + e_{ij}$$

where Y_{ij} = dependent variable, B_0 = overall intercept across all experiments (fixed effect), B_1 = linear regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable (dietary tannin concentration), s_i = random effect of

Table 2 *In vivo* experiments (Expt) included in the meta-analysis of the effect of dietary tannin level on methane emissions

Expt no.	Reference	Species	Basal feed	Tannin source*	Tannin form	Tannin level (g/kg DM)	Adaptation period	Gas sampling†	CH_4 method
16	Śliwiński et al. (2002b)	Sheep	Hay and concentrate	*Castanea* sp. (HT)	Extract	1 and 2‡	12 days	Days 19–20	RCS
17	Woodward et al. (2002)	Cattle	–	*Lolium perenne*, *Hedysarium coronarium* (CT)	Non-extracted	0 and 27.2	Not specified	Days 10–12	SF_6
18	Pinares-Patino et al. (2003)	Alpaca	–	*Lolium perenne*/*Trifolium repens*, *Lotus corniculatus* (CT)	Non-extracted	0.9 and 23.5	15 days	Days 16–19	SF_6
19	Pinares-Patino et al. (2003)	Sheep	–	*Lolium perenne*/*Trifolium repens*, *Lotus corniculatus* (CT)	Non-extracted	0.8 and 43.6	15 days	Days 16–19	SF_6
20	Woodward et al. (2004)	Cattle	–	*Lolium perenne*, *Lotus corniculatus* (CT)	Non-extracted	0 and 26.2	7 days	A total of 5 days	SF_6
21	Puchala et al. (2005)	Goat	–	*Digitaria ischaemum*/*Festuca arundinacea*, *Lespedeza cuneata* (CT)	Non-extracted	5 and 177	7 days	Day 8	RCS
22	Carulla et al. (2005)	Sheep	Ryegrass, red clover, alfalfa	*Acacia mearnsii* (CT)	Extract	0 and 25	12 days	Days 19–20	RCS
23	Beauchemin et al. (2007)	Cattle	Barley silage, concentrate	*Schinopsis* sp. (CT)	Extract	0–18.2	23 days	Days 24–26	RCS
24	De Oliveira et al. (2007)	Cattle	–	*Sorghum bicolor* silage (CT)	Non-extracted	0.2–1	10 days	Days 11–16	SF_6
25	Animut et al. (2008a)	Goat	–	*Lespedeza cuneata* (CT), *Lespedeza striata* (CT)	Non-extracted	140–151	28 days	Days 35–36	RCS
26	Animut et al. (2008b)	Goat	Sorghum, Sudan grass	*Lespedeza striata* (CT)	Non-extracted	0.3–151	9 days	Days 20–21	RCS
27	Tiemann et al. (2008a)	Sheep	Palisade grass	*Flemingia macrophylla* (CT), *Calliandra calothyrsus* (CT)	Non-extracted	0–32.8	14 days	Days 20–21	RCS
28	Grainger et al. (2009)	Cattle	Ryegrass pasture, grain	*Acacia mearnsii* (CT)	Extract	0–18	5 weeks	4 days in weeks 2/5	SF_6
29	Poungchompu et al. (2009)	Cattle	Rice straw, +/− concentrate	*Sapindus saponaria* fruit and *Garcinia mangostana* peel	Non-extracted	0 and 4.8	14 days	Day 21	GC
30	Ramirez-Restrepo et al. (2010)	Sheep	–	*Lolium perenne*/*Trifolium repens*, *Salix* spp. (CT)	Non-extracted	2.1 and 34.2	Not specified	5 days in weeks 5/11	SF_6

CT, condensed tannins; GC, gas chromatograph; HT, hydrolysable tannins; RCS, respiration calorimetry system; DM, dry matter.

*Unless tannin source is specified in brackets, the statement refers to mixed tannins.

†Time after the start of the experiment when the gas was measured/sampled for CH_4 measurement.

‡Tannin level was not directly measured, but the information was obtained from a supplier.

experiment i, b_i = random effect of experiment i on the regression coefficient of Y on X in experiment i and e_{ij} = the unexplained residual error. The variable 'experiment' was declared in the CLASS statement as it did not contain any quantitative information. In addition, an unstructured variance–covariance matrix (type = un) was declared as the random part of the model to avoid a positive correlation between intercepts and slopes (St-Pierre, 2001). Model statistics, apart from the regression equations, presented are p-value, root mean square error (RMSE) and coefficient of determination (R^2).

Data were weighted by the number of replicates or animals in each experiment (Patra, 2010) and scaled to 1 to take into consideration unequal variance among experiments. Outliers were identified by examining studentized residuals as values beyond ±3 standard deviations and subsequently removed from the data set. From the entire data set, one CH_4/digestible organic matter (OM) value originating from the *in vivo* study of Poungchompu et al. (2009) was considered as an outlier and therefore excluded. For graphical representation of the meta-analysis results, adjustments were made to the response variables to take into account the random effect of experiment. Variables being dependent on body size were standardized by relating them to metabolic body weight ($BW^{0.75}$) to counterbalance the associated variation among and within ruminant species. All data reported were transformed into the same units of measurements. Microbial count variables (both bacterial and protozoal counts) were logarithmized to allow linear relationships with the dependent variable. Additionally, the variable CH_4/digestible OM was reassessed for the *in vitro* batch cultures with a quadratic equation of the model as this model statistics resulted in lower RMSE and higher R^2 as compared to the linear model. The changes in CH_4/digestible OM were calculated by subtracting the intercept of the regression equation, i.e. the value at dietary tannins equal to 0, from each CH_4/digestible OM value, and gave them as a proportion of the intercept. As data were unbalanced concerning the completeness of data on variables, meta-analyses were performed based on the data available for individual variables.

Results

In vitro batch culture experiments

The linear regressions between dietary tannin levels and ruminal fermentation parameters from the *in vitro* batch culture experiments are presented in Table 3. Methane emission, when expressed as both ml/g substrate and ml/l total gas production, decreased (both at $p < 0.001$ in linear equations)

Table 3 Equations for linear regression of ruminal fermentation parameters on dietary tannin level (in g/kg DM) based on *in vitro* batch culture experiments

		Parameter estimates							
Response parameter	*n*	Intercept	SE intercept	p Intercept	Slope	SE slope	p Slope	RMSE	R^2
CH_4 (ml/g)	107	32.0	2.95	<0.001	−0.0751	0.0116	<0.001	5.60	0.392
CH_4 (ml/l gas)	126	157.2	12.48	<0.001	−0.267	0.0390	<0.001	19.92	0.401
Gas (ml/g)	107	181.8	63.69	ns	−0.289	0.0660	<0.001	32.59	0.239
IVOMD (mg/g)	92	665.0	40.44	<0.001	−0.507	0.1888	0.009	89.85	0.106
Total SCFA (mM)	97	62.8	6.82	<0.001	−0.0693	0.0138	<0.001	6.17	0.315
C_2 (% total SCFA)	95	64.3	3.55	<0.001	0.0061	0.00346	0.083	1.54	0.054
C_3	95	19.5	0.46	<0.001	−0.0044	0.00246	0.079	1.15	0.051
C_4	95	10.4	0.78	<0.001	−0.0005	0.00150	ns	0.67	0.002
iso-C_4	63	0.91	0.129	0.002	−0.0017	0.00070	0.016	0.24	0.116
C_5	87	1.09	0.194	<0.001	−0.0003	0.00042	ns	0.19	0.011
iso-C_5	63	0.97	0.261	0.021	−0.0018	0.00077	0.027	0.26	0.102
iso-SCFA	89	2.45	0.309	<0.001	−0.0021	0.00098	0.034	0.44	0.080
C_2/C_3	97	4.25	0.554	<0.001	0.0024	0.00086	0.006	0.38	0.129
pH	82	6.71	0.142	<0.001	−0.0001	0.00016	ns	0.07	0.000
Ammonia (mM)	84	12.3	1.97	<0.001	−0.0290	0.00732	<0.001	3.27	0.246
log bacteria (10^9/ml)	48	9.76	0.275	<0.001	0.0007	0.00020	0.001	0.06	0.263
log protozoa (10^4/ml)	80	4.67	0.082	<0.001	−0.0001	0.00022	ns	0.10	0.001

C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; *n*, number of treatment; IVOMD, *in vitro* organic matter digestibility; RMSE, residual mean square error; R^2, coefficient of determination; SCFA, short-chain fatty acids; SE, standard error; DM, dry matter.

with increasing levels of dietary tannins with R^2 of 0.39 and 0.40 respectively. Although CH_4/digestible OM also decreased ($p < 0.001$), the pattern followed a quadratic response rather than a linear relationship (Fig. 1a) with an R^2 of 0.66. Total gas production and *in vitro* ruminal OM digestibility decreased with increasing dietary tannin levels ($p < 0.001$ and $p < 0.01$ with R^2 of 0.24 and 0.11 respectively). Total SCFA decreased ($p < 0.001$) with increasing dietary tannin levels as well. Acetate (C_2) proportion of total SCFA tended to increase ($p = 0.08$), while propionate (C_3) tended to decrease ($p = 0.08$) with increasing levels of dietary tannins; therefore, the ratio of C_2/C_3 increased ($p < 0.01$). *Iso*-SCFA, *iso*-butyrate (*iso*-C_4) and *iso*-valerate (*iso*-C_5) decreased as dietary tannin levels increased ($p < 0.05$). Dietary tannins were not related to C_4 and C_5 proportions of total SCFA. Increasing levels of dietary tannins decreased ruminal ammonia ($p < 0.001$) but showed no effect on pH. Although the tannins increased bacterial counts ($p = 0.001$), there was no clear change in protozoal counts.

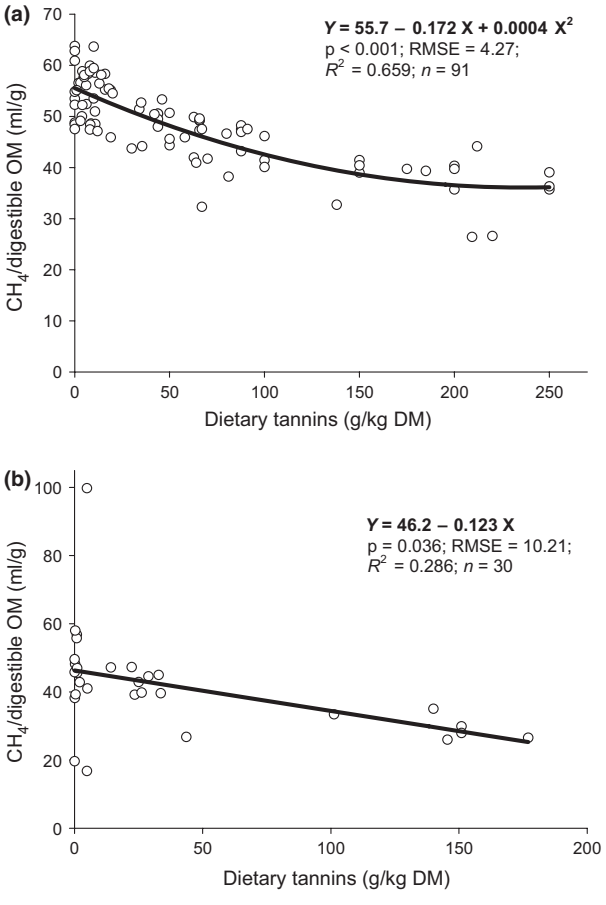

Fig. 1 Relationships between dietary tannins (g/kg dry matter) and CH_4/digestible organic matter (ml/g) from (a) *in vitro* batch culture and (b) *in vivo* experiments.

In vivo experiments

Emissions of CH_4 per unit of metabolic body weight decreased as the dietary tannins increased ($p < 0.05$, $R^2 = 0.36$; Table 4). The relationship was closer when CH_4 was expressed per unit of DM intake (DMI; $p < 0.01$, $R^2 = 0.47$). There was still a significant, though weaker, decrease in CH_4/digestible OM with increasing levels of dietary tannins ($p < 0.05$, $R^2 = 0.29$; Fig. 1b). The variation of CH_4/digestible OM values decreased with increasing tannin level. Additional dietary tannins tended to increase DMI of ruminants ($p = 0.08$). At the same time, total digestive tract OMD ($p < 0.01$, $R^2 = 0.60$), apparent crude protein digestibility (CPD) ($p < 0.001$, $R^2 = 0.78$) and neutral detergent fibre digestibility (NDFD) ($p < 0.001$, $R^2 = 0.63$) decreased. This was especially pronounced with CPD (−1.6 g/kg per g/kg extra tannins in the diet) and less so with NDFD (−1.1 g/kg). There was no significant relationship between dietary tannins and either body N retention or SCFA variables, pH, bacterial and protozoal counts across experiments. Ruminal ammonia concentration decreased with increasing levels of dietary tannins ($p < 0.01$, $R^2 = 0.56$).

Discussion

Despite the increasing experimental body reported in publications, there is, to our knowledge, still no attempt summarizing the effects of tannins on CH_4 emissions from ruminants quantitatively. This meta-analysis assists in dealing with contrasting results on such tannin effects obtained from individual experiments as it demonstrates which part of the effect is consistent across various tannin sources and experimental techniques. Tannin effects could also be compared across experimental techniques in order to assess the accuracy of *in vitro* approaches to reflect *in vivo* conditions.

Relationship between dietary tannin level and CH_4 emissions

Although individual tannins have a different biological activity in general and even slight changes in the structure can produce measurable effects (Mueller-Harvey, 2006), there seems to be an underlying general effect of tannins against methane formation as became obvious from the present meta-

Table 4 Equations for linear regression of digestive parameters on dietary tannin level (in g/kg DM) based on *in vivo* experiments

		Parameter estimates							
Response parameter	*n*	Intercept	SE intercept	p Intercept	Slope	SE slope	p Slope	RMSE	R^2
CH_4 (ml/g $BW^{0.75}$/day)	41	2.59	0.394	<0.001	−0.0063	0.00257	0.022	0.44	0.358
CH_4/DMI (ml/g)	39	30.9	3.65	<0.001	−0.109	0.0336	0.004	6.03	0.473
DMI (mg/g $BW^{0.75}$/day)	39	80.5	11.21	<0.001	0.104	0.0559	0.075	9.19	0.262
OMD (mg/g)	27	700.0	26.33	<0.001	−1.076	0.3333	0.005	40.89	0.602
CPD (mg/g)	29	637.7	49.73	<0.001	−1.644	0.5280	<0.001	40.89	0.779
NDFD (mg/g)	22	622.7	22.38	<0.001	−1.143	1.0412	<0.001	40.89	0.631
N retention (g/day)	15	3.41	1.470	0.081	0.0097	0.0097	ns	0.93	0.255
Total SCFA (mM)	24	102.1	13.55	<0.001	−0.0828	0.07438	ns	12.85	0.140
C_2 (% total SCFA)	24	67.9	2.28	<0.001	0.0023	0.01648	ns	3.01	0.002
C_3	24	19.6	2.22	<0.001	−0.0010	0.01488	ns	2.68	0.001
C_4	28	9.74	0.716	<0.001	−0.0023	0.00476	ns	0.83	0.026
isoC_4	16	1.07	0.243	0.007	0.0002	0.00061	ns	0.10	0.029
C_5	13	1.37	0.276	0.008	0.0008	0.00076	ns	0.11	0.215
isoC_5	16	1.80	0.404	0.007	−0.0009	0.00129	ns	0.22	0.086
*iso*SCFA	16	2.87	0.642	0.007	−0.0007	0.00180	ns	0.30	0.025
C_2/C_3	28	3.78	0.492	<0.001	0.0003	0.00254	ns	0.42	0.002
pH	19	6.62	0.125	<0.001	0.0001	0.00111	ns	0.12	0.087
Ammonia (mM)	28	11.4	1.89	<0.001	−0.0265	0.00828	0.005	1.31	0.564
log bacteria (10^9/ml)	15	10.4	0.28	<0.001	−0.0008	0.00171	ns	0.16	0.272
log protozoa (10^4/ml)	19	5.92	0.113	<0.001	−0.0007	0.00116	ns	0.13	0.186

C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; CPD, apparent crude protein digestibility; DMI, dry matter intake; *n*, number of treatments; NDFD, neutral detergent fibre digestibility; OMD, organic matter digestibility; RMSE, residual mean square error; R^2, coefficient of determination; SCFA, short-chain fatty acids; SE, standard error; DM, dry matter.

analysis. The present review does not attempt to differentiate between tannin sources, even not between HT and CT, but there is evidence for antimethanogenic activity for both of these two major tannin fractions (Jayanegara et al., 2011a).

Part of the CH_4 decrease with additional tannins appears to be attributable to a concomitant decline in digestibility of nutrients. The latter is the result of complexes formed by tannins with proteins and carbohydrates under ruminal pH conditions (Reed, 1995; McSweeney et al., 2001; Makkar, 2003; Mueller-Harvey, 2006). Fibre appears to interact with tannins through hydrogen bonds formed with the large number of free phenolic groups (Silanikove et al., 2001). Any reduction in fibre degradation is likely to reduce methane formation because fibrolysis delivers H_2 as a substrate for methanogenesis in forming acetate from pyruvate (Moss et al., 2000; Tavendale et al., 2005). Additionally, an increased propionate production from pyruvate actually consumes H_2 and thus should lead to even lower CH_4 amounts (Moss et al., 2000). However, the meta-analysis did not support the assumption that CH_4 mitigation by increasing dietary tannin levels is actually owing to a decrease in the acetate-to-propionate ratio. As is obvious from the present evaluation, protein degradation, and its main rumen microbial degradation product, ammonia, is closer related to extra tannins than fibre degradation (McSweeney et al., 2001). The tannin–protein interaction and its consequences have been reported in numerous other studies (Makkar, 2003; Mueller-Harvey, 2006). Consistent with the reduced protein degradation, the proportions of *iso*-C_4 and *iso*-C_5 were found to be lower in the meta-analysis of the *in vitro* batch culture experiments. These *iso*-SCFA originate from branched-chain amino acids (Owens and Bergen, 1983). The effects on protein digestion, though manifold, are unlikely to largely influence methanogenesis (e.g. Külling et al., 2001).

Apart from this indirect effect via a reduced ruminal nutrient degradation, the meta-analysis also revealed the presence of a genuine effect of dietary tannins in abating CH_4, namely in CH_4 per unit of digestible OM. This suggests that tannins may have a direct inhibitory effect on the methanogens. It has been previously reviewed that pyrogallol, gallic acid and tannic acid, which are among the monomers of tannins, are toxic for methanogens (Scalbert, 1991). Recently, Bhatta et al. (2009) reported that tannins suppressed the total population of methanogens *in vitro* by on average 12% when incubated with

130–185 g HT/kg DM and by 29% when incubated with 53–113 g mixed HT and CT/kg DM when compared to PEG-treated controls. Tavendale et al. (2005) demonstrated this tannin specifically affects *Methanobrevibacter ruminantium*, an important ruminal methanogen.

Still, the tannin effect against methane formation could be indirect. Ruminal protozoa as major H_2 producers host a certain proportion of the methanogens, and this association of protozoa and methanogens therefore contributes to CH_4 emissions (Vogels et al., 1980; Beauchemin et al., 2008). Therefore, a reduction in protozoal counts may explain effects on CH_4 emissions as well. However, the meta-analysis did not reveal a relationship between dietary tannins and protozoal counts in any type of system. Makkar (2003) as well as Patra and Saxena (2009) stated that the effects of different tannins on protozoal counts are variable, and holotrichs seem to be more susceptible to tannins than the most abundant group, the entodiniomorphs (Makkar et al., 1995; Carulla et al., 2005).

It is interesting to note that the variation in CH_4 production/digestible OM *in vivo* was very high at low levels of dietary tannins of <20 g/kg DM, whereas variability clearly decreased with increasing tannin concentrations. This might explain why experiments using low levels of tannins led to inconsistent results in terms of effects on CH_4 emissions. Probably, the influence of other dietary components masks that of tannins at low levels. Another explanation might be that low levels of dietary tannins are not sufficient to produce systematic anti-CH_4 effects at all. In turn, very high levels of dietary tannins (>200 g/kg DM) may not be efficient any more as well, as nutrient digestion is then further hampered, but this without corresponding decrease in CH_4 emissions. Accordingly, CH_4/digestible OM does not decrease anymore at these levels of dietary tannins. Tannin effects against CH_4 then would solely result from the concomitantly impaired digestibility.

Different forms of tannins (whole plants or extracted tannins) may influence the CH_4 emissions and rumen fermentation parameters differently, most probably with respect to the magnitude of the effects. In the whole plants, the presence of other components might negatively or positively influence CH_4 emissions such as fibre (Beauchemin et al., 2008), lipids (Machmüller et al., 2000), saponins (Hess et al., 2003) and essential oils (Benchaar et al., 2008). Nevertheless, it was shown that the variable 'total tannin content of plants' alone explained 55% of the total variation occurring in CH_4/digestible OM *in vitro*, which is quite considerable, as indicated by the coefficient of determination from the regression analysis (Jayanegara et al., 2011a). To our knowledge, there is no study that has attempted to directly compare between tannins present in whole plants and those extracted from these plants provided at the same tannin level in relation to CH_4 emissions. This question is important to be addressed in order to know which form is more reliable and efficient in mitigating CH_4 and at the same time has fewer adverse effects on nutrient digestibility and productivity of the animals.

Suitability of *in vitro* methods to predict *in vivo* relationships of dietary tannins and CH_4 emissions

Comparisons between different methods in determining the tannin effects are possible by presenting CH_4 in the same units of measurement. Three units might be suitable for such a comparison: (i) CH_4 per unit of dietary DM (ml/g), (ii) CH_4 per unit of dietary digestible OM (ml/g) and (iii) the proportional change in CH_4/digestible OM (in percentage; cf. Fig. 2) by taking the intercept of the regression equation as the reference value.

In contrast to *in vitro* batch culture methods, in the *in vivo* system, media continuously flow out and there is direct absorption from the rumen. In the literature, the relationship between CH_4 produced *in vivo* and *in vitro* has been described as to be poor (R^2 = 0.26; Moss and Givens, 1997), leading to statements that it is extremely difficult to extrapolate from *in vitro* measurements to *in vivo* situations in

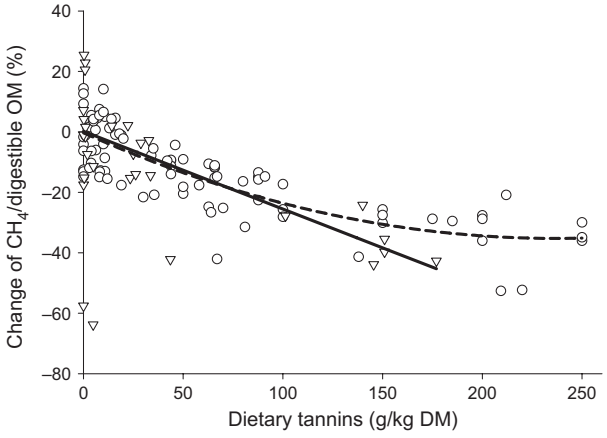

Fig. 2 Comparison between *in vitro* batch culture experiments (○, dashed regression line) and *in vivo* experiments (▽, full regression line) concerning the influence of dietary tannins (g/kg dry matter) on the change in CH_4/digestible organic matter (%).

ruminants, or to field conditions (Flachowsky and Lebzien, 2009), and certain limitations of the *in vitro* batch culture technique to simulate *in vivo* conditions are known (Krishnamoorthy et al., 2005). Still, other studies also reported that the *in vitro* batch culture technique may accurately estimate CH_4 emissions *in vivo* (Blümmel et al., 2005; Bhatta et al., 2007). From the present evaluation, it seems that, applied on a specific dietary measure, the applicability especially of the *in vitro* batch culture technique is given. Indeed, the intercepts of CH_4 production per unit of DM (i.e. substrate without tannins) from *in vitro* batch culture and *in vivo* experiments were similar, with 32.0 and 30.9 ml/g DM respectively. However, when CH_4 was presented as CH_4/digestible OM, the intercepts were less similar with 55.7 and 46.2 ml/g. Furthermore, up to a level of approximately 100 g tannins/kg DM, the *in vitro* batch culture system appeared to predict the changes taking place in CH_4 emissions accurately (Fig. 2). Only at even higher tannin levels, this system seemed to underestimate the true reduction in CH_4 formation with extra tannins even though the trend towards lower CH_4 with higher tannin levels was still obvious. It should be also noted that the regressions were calculated from different ranges of dietary tannin levels (cf. Fig. 2).

Conclusions

Across a large number of experiments, increasing the level of dietary tannins basically leads to a clear decrease in ruminal CH_4 emissions. This is helpful as the search for antimethanogenic sources of tannins can put more emphasis on additional economically relevant criteria such as satisfactory palatability and low adverse effects on animal performance. However, reliable and distinguishable effects of tannins can be expected only from levels >20 g/kg DM, a threshold often not exceeded in current commercial feed supplementation with tannins. Measurements with *in vitro* batch culture systems could provide an inexpensive starting point for screening of potential CH_4 tanniferous inhibitors (up to <100 g tannins/kg DM), whereas individual promising substrates then need to be investigated in detail *in vivo*.

Acknowledgements

The first author is grateful to Indonesian Minister of National Education for providing a scholarship to conduct the PhD study at ETH Zurich, Switzerland.

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