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Comparison of methane production between C3 and C4 grasses and legumes

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A B S T R A C T

A meta-analysis was conducted to compare effects of C4 and C3 grasses as well as warm and cold climate legumes on CH_4 production of ruminants. For this purpose, a database was built using 22 *in vivo* studies containing 112 observations with 58 C3 grasses, 28 C4 grasses, 26 cold legumes and 12 warm legumes. Neutral detergent fibre (NDF), crude protein (CP) and total tract organic matter (OM) digestibility ranged from 415 to 753 g/kg DM *versus* 361 to 754 g/kg DM, from 24 to 254 g/kg DM *versus* 44 to 314 g/kg DM and from 0.51 to 0.71 *versus* 0.56 to 0.83 for C4 and C3 grasses, respectively. The NDF, CP and total tract OM digestibility ranged from 441 to 690 g/kg DM *versus* 252 to 684 g/kg DM, from 93 to 236 g/kg DM *versus* 141 to 269 g/kg DM and from 0.42 to 0.57 *versus* 0.38 to 0.79 for warm and cold legumes, respectively. Relationships between CH_4 production and forage characteristics were analysed by analysis of covariance. For grasses, the main factors tested as fixed effects were NDF and CP content of the diet, total tract OM digestibility, intake, animal species, forage family and random trial effect. For legumes, tannin level was included in the model. Results indicate that ruminants fed C4 grass produced 17% more CH_4 as L/kg OM intake ($P<0.05$) compared to those fed C3 grass. Animals fed warm legumes produced 20% less CH_4 ($P<0.05$) than those fed C4 grasses. In contrast, no difference in CH_4 production between C3 grasses and cold legumes. Use of some legumes in warm climates could be a strategy to reduce CH_4 emissions by ruminants.

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Abbreviations: DM, dry matter; DOM, digestible OM; LW, live weight; NDF, neutral detergent fibre; NDFf, forage neutral detergent fibre; OM, organic matter; OMd, OM apparent digestibility.

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1. Introduction

Diet composition and intake are main factors affecting CH_4 production by ruminants. Ruminants fed forages rich in structural carbohydrate produce more CH_4 than those fed mixed diets containing higher levels of non-structural carbohydrates (Sauvant and Giger-Reverdin, 2009). Forages are often the main ingredient in ruminant diets in the tropics. In these regions, feed resources (*i.e.*, grasses, legumes, tree foliage) differ from those of temperate regions due to their chemical and structural composition and digestibility (Leng, 1990; Assoumaya et al., 2007). Tropical grasses generally use C4 metabolic pathways for photorespiration whereas most temperate grasses use C3 carbon fixation. Often, the C4 metabolic pathway leads to a higher rate and degree of deposition of lignin in plant tissues, a factor which can alter voluntary intake and digestion (Wilson, 1994), although there are exceptions. Some tropical forages, such as legumes, shrubs and tree foliages may contain secondary metabolites such as condensed tannins and saponins that alter rumen methanogenesis (Jouany and Morgavi, 2007).

It has been reported that ruminants fed tropical forages produce more CH_4 than ruminants fed temperate forages (McCrabb and Hunter, 1999). Also, the presence of condensed tannins in legume, tree and shrub foliage has been found to lower CH_4 emissions (Waghorn, 2008) mainly due to inhibition of methanogens in the rumen (Martin et al., 2010). However, studies based on direct *in vivo* comparison of C4 *versus* C3 grass, or of legumes grown in warm ('warm' legume) *versus* cold environments ('cold' legume) have been rare due to geographical constraints.

Our objective was to compare CH_4 emissions of ruminants fed C4 *versus* C3 grass, and warm *versus* cold legumes using a meta-analysis approach with consideration for explanatory factors such as dietary fibre content, total tract digestibility and intake.

2. Materials and methods

2.1. Publications selection and codification for the database

The database used contained 112 treatments extracted from 22 scientific publications. To be included, a publication had to contain data on forage composition, feed intake, digestibility and CH_4 emission measurements. Only all forage diets were included and cereal forages, such as corn and sorghum, were not included. Forages were classified into grasses and legumes. A grass was assigned as C4 when it had a C4 metabolic pathway, which is characteristic of tropical grasses, and it was harvested in the tropics. A grass was assigned as C3 when it had a C3 metabolic pathway, which is characteristic of temperate grasses, and it was harvested in temperate environments. As all legumes have C3 metabolic pathways, they were classified into 'warm' and 'cold' types. A warm type was defined as being grown mainly in a tropical environment, and occasionally in a temperate environment during the warm season. A cold type was defined as being grown in a temperate environment. C4 grasses and warm legumes were defined as tropical forages, and C3 grasses and cold legumes were defined as temperate forages. This classification resulted in a distribution of studies among forage types of C3 grass ($n = 58$), cold legume ($n = 14$), C4 grass ($n = 28$) and warm legume ($n = 12$). As CH_4 production has been shown to be affected by tannins (Waghorn, 2008), legumes were coded according to their tannin concentration. Three levels, being high, medium and low were defined on the basis of literature data. High concentrations corresponded to more than 300 g tannin/kg DM, medium concentration included legumes with 100–300 g tannin/kg DM and low included those legumes containing less than 100 g tannin/kg DM. Animal species were coded as large (*i.e.*, cows, heifers, calves; $n = 62$) and small (*i.e.*, sheep, goats; $n = 50$) ruminants. The methods used to measure CH_4 were coded as: calorimetric chamber, SF_6 gas tracer technique and gas bag technique.

2.2. Calculations

Some estimates were made of organic matter (OM) level of the diet and OM digestibility when data were lacking in the publications. The OM content of diet and OM digestibility were estimated as DM multiplied by 0.9 and DM digestibility multiplied by 1.05, respectively, according to tables of feed values in temperate and tropical countries. The CH_4 production was expressed in litres or per unit of DM intake (kg), (L/kg DM intake), OM intake (kg) (L/kg OM intake), or digested OM (L/g DOM).

2.3. Statistical analyses

The meta-analysis followed recommendations of Sauvant et al. (2008) using Minitab (2007). Variation in predictability of CH_4 production attributable to a number of variables including OM intake, neutral detergent fibre (NDF) intake, digestible OM intake, forage NDF (NDFf) and OM digestibility (OMd) was examined. Level of intake was considered using the OM intake/liveweight $(LW)^{0.9}$ (Poppi et al., 1990) as covariates. Predictions of CH_4 production were similar to those obtained with LW as a covariate. Several qualitative factors were accounted for, such as type of forage (C3 grass, cold legume, C4 grass, warm legume), animal species (large and small ruminants), CH_4 measurement method (calorimetric chamber, SF_6 gas tracer technique, gas bag technique) and tannin level (low, medium and high). Moreover a random effect of study ($n = 22$) was also included in the model.

Table 1
Statistical description of the chemical composition for each type of forage or animals in the database.

	Parameter	Grasses		Legumes	
		C3 type	C4 type	Cold	Warm
	n	58	28	14	12
NDF (g/kg DM)	Mean	557.3	646.4	447.2	568.2
	SD	84.81	97.89	114.67	98.94
	Minimum	361.0	415.0	252.0	441.0
	Maximum	754.0	753.0	684.0	690.0
CP (g/kg DM)	Mean	169.5	110.3	198.6	156.6
	SD	55.94	62.80	38.40	56.91
	Minimum	44.0	24.0	141.0	93.0
	Maximum	314.0	254.0	269.0	236.0
OM intake (g/kg LW)	Mean	19.8	17.7	17.8	18.6
	SD	5.48	6.98	6.00	4.57
	Minimum	10.6	7.4	9.3	7.3
	Maximum	33.9	31	28.8	25.2
OM intake (g/kg $LW^{0.9}$)	Mean	34.4	27.4	31.4	25.6
	SD	8.27	11.81	9.30	6.44
	Minimum	15.7	10.7	13.8	10.7
	Maximum	48.9	56.9	51.1	36.3
OM apparent total tract digestibility	Mean	0.70	0.62	0.63	0.48
	SD	0.075	0.057	0.010	0.053
	Minimum	0.56	0.51	0.38	0.42
	Maximum	0.83	0.71	0.79	0.57

Those variables that accounted for the majority of the predictability of CH_4 production, and were adequately represented among studies, were selected for the final model and included the factors: random study effect, forage, level of intake (OM intake/$LW^{0.9}$), OMd and NDFf. The statistical analysis model of the database was:

$$y_{ijk} = \mu + a_i + b_j + \alpha \mathrm{OM}_{\mathrm{intake}} + \beta \mathrm{OM}_{\mathrm{digestibility}} + \delta \mathrm{NDF}_{\mathrm{content}} + \varepsilon_{ijk}$$

where y_{ijk} is the CH_4 production, a_i is the ith random trial effect ($i = 1, \ldots, 22$), b_j is the jth forage type ($j = 1, \ldots, 4$), α is the coefficient associated with the covariable OM intake, β is the coefficient associated with the covariable OM digestibility, δ is the coefficient associated with the covariable NDF content and ε_{ijk} is the residual error.

When a treatment was an outlier (*i.e.*, beyond 3 standard deviations from the adjusted mean) for a character or a specific relationship, it was removed from the statistical analysis.

An analysis of variance was completed on legumes to estimate tannin level effect on CH_4 production. Studies and tannins levels were considered as fixed effects. MOI/$LW^{0.9}$, OMd and NDFf were used as covariates in the model:

$$y_{ijk} = \mu + a_i + b_j + \alpha \mathrm{OM}_{\mathrm{intake}} + \beta \mathrm{OM}_{\mathrm{digestibility}} + \delta \mathrm{NDF}_{\mathrm{content}} + \varepsilon_{ijk}$$

where y_{ijk} is the CH_4 production of legumes, a_i is the ith random trial effect ($i = 1, \ldots, 7$), b_j is the jth tannin level ($j = 1, \ldots, 3$), α is the coefficient associated with the covariable OM intake, β is the coefficient associated with the covariable OM digestibility, δ is the coefficient associated with the covariable NDF content and ε_{ijk} is the residual error.

3. Results

3.1. Database characteristics

Studies included in the model are in Table 1, with at least 3 studies contributing to the mean values. Data included 50 studies in small ruminants (8 goats and 42 sheep) and 62 studies in large animals (34 cows, 21 heifers, 7 calves). Tropical forages are underrepresented relative to temperate forages, and grasses have been studied more than legumes. Whatever the origin of forage, the variation in chemical composition and digestibility is broad and representative of forage diversity.

3.2. Methane production and diet

Those measured components that had the closest relationship to aspects of methane production included:

$$\mathrm{CH_4 \quad L/kg \quad DMintake} = -4.2(\pm 11.3) + 39.2(\pm 9.2) \times \mathrm{OMd} + 21.1(\pm 11.4) \times \mathrm{NDFf} - 0.15(\pm 0.06) \times \mathrm{OM \quad intake/LW^{0.9}} \quad (n = 115, \quad R^2 = 0.77, \quad \mathrm{rsd} = 4.1, \quad \mathrm{P} < 0.0001)$$

Table 2
Effect of forage type on CH_4 production, expressed as L/kg dry matter (DM) intake, L/kg organic matter (OM) intake, L/kg digested OM (DOM) or L/100 kg live weight (LW).

	CH_4 (L/kg DM intake)	CH_4 (L/kg OM intake)	CH_4 (L/kg DOM)
Grasses			
C3 type	30.0b	33.1b	52.1b
C4 type	33.7c	38.8c	57.7b
Legumes			
Cold	30.1b	33.7bc	52.4b
Warm	25.9a	27.2a	40.7a
SEM	1.77	1.79	2.89
R^2	0.77	0.84	0.76
P	0.001	0.001	0.001

Mean values within column with no common letter (a–c) differ (P<0.05). Effects used in the model were: experiment, animal species, diet, forage NDF content, OM apparent total tract digestibility and OM intake/$LW^{0.9}$.

$$CH_4 \quad L/kg \quad OMintake = 0.9(\pm 11.4) + 39.8(\pm 3.1) \times OMd + 18.9(\pm 11.49) \times NDFf - 0.2(\pm 0.06) \times OM\,intake/LW^{0.9}$$
$$(n = 115, \quad R^2 = 0.84, \quad rsd = 4.1, \quad P < 0.0001)$$

$$CH_4 \quad L/kg \quad OMD = 34.1(\pm 9.6) + 40.4(\pm 15.2) \times NDFf - 0.3(\pm 0.09) \times OM \quad intake/LW^{0.9}$$
$$(n = 115, \quad R^2 = 0.76, \quad rsd = 6.6, \quad P < 0.0001)$$

However, the accuracy of the models differed depending on how CH_4 production was expressed, with accuracy higher when CH_4 production was expressed as L/kg OM intake. Whatever the mode of expression of CH_4, there were no interactions between class of forage and NDFf or between class of forage and OMd. As expected, CH_4 production increased with increasing NDF content and OMd of the diet, and decreased with declining intake.

Methane production was higher for animals fed C4 grasses (Table 2) compared to those fed C3 grasses regardless of how CH_4 production was expressed. Methane production was lower for animals fed warm legumes compared to those fed cold legumes.

A specific statistical analysis of legumes ($n = 25$, $R^2 = 0.92$, rsd = 2.6, P=0.01), indicated that CH_4 production (L/OM intake) was lower for animals fed high tannin legumes (21.8 L/kg MOI *versus* 27.8 L/kg MOI) compared to those fed low tannin legumes. The same model ($n = 25$, $R^2 = 0.80$, rsd = 5.6, P=0.01) resulted in estimates of 37.2 L/kg DOM intake *versus* 52.2 ± 2.7 L/kg DOM intake for high tannin legumes *versus* low tannin legumes.

4. Discussion

The main objective was to compare CH_4 production in ruminants fed tropical or temperate forages. Some publications support the hypothesis that tropical C4 grasses are more methanogenic than temperate C3 grasses (Kurihara et al., 1999; McCrabb and Hunter, 1999; Ulyatt et al., 2002). To our knowledge, only Margan et al. (1998) directly compared of CH_4 production between two tropical forages and a temperate forage in the same study. Most comparisons are based on data obtained under different situations. Other conclusions come from inference of the positive correlation between forage fibre level and CH_4 production (Blaxter and Wainman, 1964; Moe and Tyrrell, 1979). As tropical forages are usually higher in fibre than temperate forages, many models assign higher estimates of CH_4 production to them. However, this criterion does not consider the wide variability in fibre contents that exist within tropical and temperate forage groups with broad overlaps between these forage classes.

Our meta-analysis compared tropical and temperate forages at the same level of fibre, digestibility and level of intake. It demonstrated that CH_4 production for C4 grasses was 10–17% higher, depending on the unit, compared to C3 grasses. In contrast, CH_4 production from legumes grown in warm climates was 7–22% lower than for legumes grown in cold climates. These values can be compared with those of Margan et al. (1998) who found that sheep fed C4 grasses produced 23% more CH_4 (MJ/MJ intake) than sheep consuming a C3 grass, but only two C4 grasses were studied. Differences between C4 and C3 grasses occurred when CH_4 production was expressed as L/OM intake and L/DOM intake. These differences were not due to a lower digestibility for C4 grasses because the forages were adjusted for digestibility by inclusion of this variable as a covariate in the model. While intake and NDF content were included as covariables in the model, differences in CH_4 production between C4 and C3 grasses persisted. However, the nature of the fibre differs between tropical and temperate forages (Wilson, 1994) with C4 grass fibres tending to be more lignified and more resistant to physical and microbial digestion than those of C3 grasses. At the same level of digestibility, tropical grasses also have a longer retention time in the rumen (Assoumaya, 2007). This difference in rumen retention between C4 and C3 grasses may account for a portion of the difference in CH_4 production. Others have shown that increasing retention time of forages in the rumen can lead to increased CH_4 production (Pinares-Patino et al., 2003).

The VFA profile could help to explain differences in CH_4 production between C3 and C4 grasses. Unfortunately, to our knowledge, neither *in vivo* nor *in vitro* experiments have been completed to compare VFA profiles between C3 and C4 grasses. Data from the comparison of old and young re-growth of C4 grass, which approximates digestion of C3 grass (Assoumaya, 2007), could be of interest. Assoumaya (2007) compared digestion of the C4 plant *Panicum maximum* between 14 and 56 days of re-growth and found a decrease in propionate relative to total VFA, while acetate increased with advancing maturity. Rumen fermentation profiles could therefore partially explain differences between temperate and tropical grasses. However, variation of CH_4 production among the 5 types of forage is lower than that in mixed diets where the proportion of forage and concentrate varies (Johnson and Johnson, 1995). This likely reflects the small change in the ratio of propionate/acetate ratios in forages fed to ruminants in contrast to mixed diets (Johnson and Johnson, 1995).

Compared to grasses, legumes have at least two characteristics which could influence OM digestion in the rumen and, consequently, CH_4 production. The highly lignified cell walls and the presence of secondary metabolites such as tannins decrease cell wall digestion in legumes. Notwithstanding, there is high variability among legumes, in particular regarding the presence of secondary metabolites such as tannins which are more common in tropical *versus* temperate legumes (Waghorn, 2008). In our database, the legumes highest in tannin were those of tropical origin, a factor that could account for the lower CH_4 production in warm *versus* cold legumes. Studies conducted *in vitro* (Bekele et al., 2009; Soliva et al., 2008; Tiemann et al., 2008) have shown that a large portion of the variability of CH_4 production in legumes can be accounted for by the presence or absence of secondary metabolites. In temperate conditions, fresh lucerne has been shown to produce less CH_4 than grasses (McCaughey et al., 1999), whereas clover did not (Van Dorland et al., 2007). Similarly, studies with temperate legumes found high variability in CH_4 production in ruminants depending on the presence or absence of secondary metabolites (Waghorn et al., 2002; Waghorn, 2008; Beauchemin et al., 2009; Dewhurst et al., 2009).

5. Conclusions

When fed to ruminants, it is clear that C4 grasses produce more CH_4 than C3 grasses, that legumes from warm climates produce less CH_4 than legumes from cold climates, and that legumes produce less CH_4 than grasses.

The relative scarcity of data on CH_4 production from ruminants fed tropical forages (*i.e.*, C4 grasses and warm legumes) suggests a need for additional studies. Results of this meta-analysis should be confirmed by coordinated experiments in temperate and tropical environments with the same animal genotype (*i.e.*, tropical *versus* temperate) fed both tropical and temperate forages.

Conflict of interest statement

None.

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