Do typical alpine forage plants have the potential to mitigate methane production by ruminants?

A. Jayanegara[1], M. Kreuzer[1], F. Leiber[1]
[1]ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland
Corresponding author: Florian Leiber. E-mail: fleiber@ethz.ch

Abstract

A collection of 17 different alpine forage plants (2 grasses, 8 non-leguminous herbs, 3 leguminous herbs and 4 tree species) were investigated with an *in vitro* fermentation assay for their ruminal methane production potential. All samples were harvested twice in early July of the years 2009 and 2010 from sites in the eastern Swiss Alps in the canton of Grisons. Samples were analyzed for nutrient contents and phenolic compounds. In each year, samples were incubated in four replicates for 24 h with the Hohenheim Gas Test system using bovine rumen fluid. Total gas production and gas composition were measured immediately after incubation. The results showed that almost all alpine plants investigated had lower *in vitro* ruminal methane emission potential compared to hay standard. Except for *Castanea sativa*, methane emission potential of the investigated plants varied less than expected. However, several plants proved to concomitantly express a comparably low methane emission potential and high nutritional value like *Alchemilla xantochlora* and *Sambucus nigra*.

Keywords: alpine plants, methane, nutrient, phenol, rumen

Introduction

Vegetation on the Alps is an important feed resource for livestock production in the respective regions during summer period. Rapid growth of biomass at the beginning of summer followed by a fast decline in growth and forage quality are among the main characteristics of the vegetation (Brühlmann & Thomet, 1991). Studies on the influence of alpine vegetation on productivity (Leiber *et al*., 2004) and product quality (Leiber *et al*., 2005) of ruminants have been previously conducted. However, no studies so far have attempted to assess the methane (CH_4) production potential of the plants after being ingested by ruminants. This is important to be assessed since the CH_4 accumulation in the atmosphere has gained more attention in recent years, and all related sectors are pushed to minimize emissions. Plant secondary compounds in the alpine vegetation are assumed to modulate rumen processes with significance for the product quality (Leiber *et al*., 2005). The same factors could also influence ruminal CH_4 production. Therefore, the present study is aimed to investigate CH_4 production potential of some typical plants on the Alps under *in vitro* rumen environment.

Material and methods

Samples from 17 plant species were collected in early July 2009 and 2010 from three sites of the Alps in South-Eastern Switzerland (canton of Grisons), i.e. Misox valley, Rhine forest (Sufers) and Albula valley at altitudes of 800, 1400 and 1800-2300 m a.s.l., respectively. The plants consisted of 2 grasses, 8 non-leguminous herbs, 3 leguminous herbs and 4 tree species (leaves and flowers). After collection, all plant samples were stored at 4°C overnight, oven dried at 60°C for 24 h and ground to pass a 1 mm sieve. Analysis of plant chemical composition included proximate, detergent and phenolic compound determinations. Plant samples were incubated with buffered-rumen fluid *in vitro* (in four replicates each year) using the Hohenheim Gas Test (HGT) apparatus (Menke and Steingass, 1988), conducted for the harvests of 2009 and 2010. Hay and concentrate standards were also incubated. Rumen fluid was obtained from a fistulated Brown Swiss cow before the morning feeding. After 24 h of incubation, the gas produced during fermentation was recorded and sampled for gas composition determination, including CH_4 using a gas chromatograph. Ammonia and short-chain fatty acids (SCFA) in the fermentation fluid were determined and microbial populations (bacteria and protozoa) were counted. *In vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were calculated using standard equations. Data were subjected to analysis of variance (mixed model), followed by Tukey's test for multiple comparisons among plants using SAS software version 9.2.

Results and discussion

Incubation of hay and concentrate standards resulted in CH_4/total gas values of 161 (±25.3) and 182 (±25.1) ml/l, respectively. These data, especially from hay standard, are important reference values for determining the CH_4 mitigating potential of each plant. The values are closely similar to CH_4/total gas of the control substrate (90% hay) in other studies, i.e. 174 ml/l (Bodas *et al*., 2008). Within the grass species, *Poa alpina* produced lower CH_4/total gas compared to *Nardus stricta* ($P<0.05$; Table 1). Further, *P. alpina* produced 14.3% less CH_4/total gas compared to the hay standard while *N. stricta* produced 9.3% more. This might be explained by the lower NDF content of *P. alpina* compared to *N. stricta*. Dietary cell-wall content and ruminal CH_4 emission are considered to be positively correlated since fiber degradation results in large amounts of hydrogen which is utilized by the methanogens for CH_4 formation (Machmüller *et al*., 2003). Further, *P. alpina* was superior to *N. stricta* due to higher values of total gas production, IVOMD, ME and total SCFA ($P<0.05$). The poor quality of *N. stricta* was in agreement with the low *in vivo* digestibility of alpine swards dominated by *N. stricta* (Berry *et al*., 2002). Therefore, this grass is hardly accepted by grazing ruminants (Fischer & Wipf, 2002).

Table 1: Chemical composition of plants and *in vitro* fermentation variables (n = 8)

Plant species	CP	EE	NDF	TP	NH_3	Total SCFA	Total gas	CH_4/gas	IVOMD	ME
	g/kg dry matter				mM	mM	ml	ml/l	mg/g	MJ/kg
Grasses										
Nardus stricta	116	6	753	8	10.6^{cde}	69.0^{b}	30.6^{b}	176^{d}	525^{b}	7.30^{b}
Poa alpina	139	18	540	20	9.9^{bcde}	88.0^{cde}	46.6^{fgh}	138^{bc}	698^{ghi}	9.99^{fg}
Non-leguminous herbs										
Achillea millefolium	191	16	370	23	11.0^{e}	87.0^{cde}	38.4^{cd}	140^{bc}	691^{fghi}	9.10^{de}
Alchemilla xanthochlora	156	20	256	54	8.3^{abc}	94.1^{de}	47.9^{gh}	134^{bc}	736^{i}	10.34^{fg}
Carum carvi	140	16	359	21	10.9^{de}	91.2^{de}	44.2^{efgh}	152^{bcd}	709^{hi}	9.57^{ef}
Chrysantemum adustum	91	14	412	20	8.8^{abcde}	88.4^{cde}	42.5^{defg}	141^{bc}	648^{defg}	8.91^{cde}
Crepis aurea	139	33	322	24	11.0^{e}	96.5^{e}	49.9^{h}	148^{bcd}	744^{i}	10.81^{g}
Plantago atrata	110	12	444	26	7.2^{a}	77.2^{bcd}	38.7^{cde}	157^{bcd}	621^{cde}	8.46^{cd}
Rhinanthus alectorolophus	143	22	307	29	8.8^{abcde}	92.0^{de}	46.9^{gh}	146^{bcd}	725^{hi}	10.13^{fg}
Rumex arifolius	122	16	409	38	8.2^{ab}	86.1^{bcde}	40.1^{de}	142^{bc}	637^{def}	8.82^{cde}
Leguninous herbs										
Anthyllis vulneraria	131	14	372	25	8.6^{abcd}	91.6^{de}	40.4^{de}	160^{cd}	674^{efgh}	8.91^{cde}
Hedysarum hedysaroides	223	18	319	69	8.7^{abcd}	77.1^{bcd}	29.5^{b}	160^{cd}	568^{bc}	8.18^{c}
Trifolium badium	146	14	321	43	8.3^{abc}	88.5^{cde}	38.8^{cde}	153^{bcd}	650^{defg}	8.78^{cde}
Trees										
Castanea sativa	141	19	402	112	7.4^{a}	44.4^{a}	6.5^{a}	24^{a}	305^{a}	5.13^{a}
Fraxinus excelsior	160	14	408	22	7.5^{a}	71.7^{bc}	34.4^{bc}	150^{bcd}	606^{cd}	8.27^{cd}
Sambucus nigra[1]	243	35	218	37	14.6^{f}	85.8^{bcde}	39.6^{cde}	142^{bc}	712^{hi}	10.03^{fg}
Sambucus nigra[2]	247	32	260	43	16.5^{f}	95.3^{e}	41.0^{def}	129^{b}	712^{hi}	10.21^{fg}
SEM	11.0	1.9	30.0	5.9	0.32	1.68	0.89	3.3	9.4	0.122
P-value	na	na	na	na	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CP, crude protein; EE, ether extract; IVOMD, *in vitro* organic matter digestibility; ME, metabolizable energy; na, not applicable; NDF, neutral detergent fiber; SCFA, short-chain fatty acids; SEM, standard error of the mean; TP, total phenols

Mean values in the same column without common superscript are significantly different at $P<0.05$

[1] leaf; [2] flower

Within the non-leguminous herbs, all plants produced lower values of CH_4/total gas than that of hay standard. The lowest was in the incubation of *Alchemilla xanthochlora*, which resulted in 16.8% lower of CH_4/total gas vs hay standard. This might be related to the relatively high content of phenolic compounds in the plant, particularly hydrolysable tannins (HT; data not shown). The effect of HT in reducing ruminal CH_4 emission has been previously demonstrated (Bhatta *et al*., 2009). However, no difference occurred for CH_4/total gas across all non-leguminous herbs. Total gas production was highest when incubating *Crepis aurea*, followed by *A. xanthochlora* and *Rhinanthus alectorolophus*.

A closely similar pattern was also obtained for IVOMD and ME. For the leguminous herbs, CH_4/total gas was similar across all three leguminous herbs and almost similar to that of hay standard. No significant difference was found between leguminous herbs in terms of NH_3. Incubation of *Hedysarum hedysaroides* resulted in significantly lower total gas production and IVOMD than the other two plants within this functional group ($P<0.05$). The low variability of CH_4 emissions from the herbaceous species (both non-leguminous and leguminous) in the present study was in contrast to the expectations.

Within tree species, *Castanea sativa* produced extremely low CH_4/total gas (85.1% lower vs hay standard) as well as total gas, IVOMD and ME values compared to all other plants ($P<0.05$). The plant contained the highest TP and HT across all plants investigated here. Although this plant has been commonly fed to ruminants in former times, the fermentation results indicated a strongly limited forage quality. Corresponding to the high CP contents, ruminal NH_3 concentrations were higher after incubation of both *Sambucus nigra* flowers and leaves compared to all other species across the different functional groups ($P<0.05$). This proved that *S. nigra* has a good nutritional quality and fits into the range of the herbs. This plant had also a comparably low CH_4 emission across all plants, except compared to *C. sativa*, and produced 11.8% and 19.9% less CH_4/total gas than the hay standard for the leaves and flowers, respectively.

Conclusions

Almost all alpine plants investigated had lower *in vitro* ruminal CH_4 emission potential compared to hay standard. However, the emission potential among plants varied less than expected. The nutritive value was especially high for *A. xanthochlora* and *C. aurea* among the herbaceous plants and *S. nigra* among the trees. Particularly *A. xanthochlora* is interesting because in addition to its high quality, it produced the lowest CH_4 emission among the herbs. This was similar for *S. nigra*. Although *C. sativa* appeared to be a very effective CH_4 inhibitor, the plant is hardly digested and fermented in the rumen *in vitro*, thus indicating a low feeding value.

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