Research Article

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Nutrient and energy content, *in vitro* ruminal fermentation characteristics and methanogenic potential of alpine forage plant species during early summer

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Abstract

BACKGROUND: Plants growing on alpine meadows are reported to be rich in phenols. Such compounds may affect ruminal fermentation and reduce the plants' methanogenic potential, making alpine grazing advantageous in this respect. The objective of this study was to quantify nutrients and phenols in Alpine forage grasses, herbs and trees collected over 2 years and, in a 24 h *in vitro* incubation, their effects on ruminal fermentation parameters.

RESULTS: The highest *in vitro* gas production, resulting in metabolisable energy values around 10 MJ kg^{-1}, were found with *Alchemilla xanthochlora* and *Crepis aurea* (herbaceous species) and with *Sambucus nigra* leaves and flowers (tree species). Related to the amount of total gas production, methane formation was highest with *Nardus stricta*, and lowest with *S. nigra* and *A. xanthochlora*. In addition, *Castanea sativa* leaves led to an exceptional low methane production, but this was accompanied by severely impaired ruminal fermentation. When the data were analysed by principal component analysis, phenol concentrations were negatively related with methane proportion in total gas.

CONCLUSION: Variation in methane production potential across the investigated forages was small. The two goals of limited methane production potential and high nutritive value for ruminants were met best by *A. xanthochlora* and *S. nigra*.

Keywords: mountain pasture; tree leaf; ruminant; phenol; methane; ammonia

INTRODUCTION

Grasslands in the mountainous region of the Alps provide an important feed resource for ruminant livestock that complements lowland livestock systems during the period of summer grazing. Alpine pastures consist of grasses, herbs, shrubs and trees, and often have a high biodiversity.[1,2] The herbaceous vegetation is characterised by rapid growth of biomass at the beginning of summer, followed by a fast decline in growth and quality after a short period of time.[1,3] There have been a number of studies on the utilisation of alpine swards as feed sources for ruminants including assessment of nutrient and energy utilisation and conversion by dairy and beef cattle.[4,5] Focus has also been put on the influence of grazing alpine swards on the quality of dairy products by measuring the effects on milk protein,[6] fat[7,8] and sensory quality.[9,10] A clear advantage of alpine milk is its elevated content of *n*-3 fatty acids and conjugated linoleic acids.[7,8] However, only a few studies reported nutritional values of individual mountain forage species.[3,11–13]

Besides their ecological importance as habitat for a biodiverse flora and fauna,[2,14] mountain pastures are reported to consist of plants containing relative high amounts of phenols[15] and thus may have methane (CH_4) mitigating potential.[16] However, this conclusion in the context of alpine ruminant feeding still awaits confirmation. Therefore, the objectives of the present study were to collect samples of various typical and abundant alpine forage plants[3,7,17] from different functional groups, to determine their nutritive value, their composition of different phenolic fractions and to test their effect on ruminal fermentation parameters, particularly methanogenesis.

EXPERIMENTAL

Experimental plants

In early July of the years 2009 and 2010, samples of a total of 17 forage plant species were collected from three different currently grazed alpine sites (Misox valley, Rhine forest (Sufers) and Albula valley at altitudes of 800, 1400 and 1800–2300 m above sea level, respectively) located in south-eastern Switzerland (canton of Grisons; Table 1). The time point was chosen in order to evaluate the plants when most were in the flowering stage. These

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Table 1. Chemical composition of the experimental plants collected in 2009 and 2010 (g kg^{-1} dry matter; $n = 2$ per year)

				Crude protein		Ether extract		NDF		ADF		ADL	
No.	Plant species	Site	Phenological stage	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
	Grasses												
1	*Nardus stricta*	Albula	Bu	118	113	3	9	741	764	325	362	49	46
2	*Poa alpina*	Albula	Fl	130	148	16	19	501	578	232	269	56	48
	Non-leguminous herbs												
3	*Achillea millefolium*	Misox	Bu	215	167	15	17	393	347	298	279	111	70
4	*Alchemilla xanthochlora*	Albula	Fr	162	149	19	20	230	281	177	240	40	34
5	*Carum carvi*	Sufers	Fr	128	151	16	15	375	343	315	296	67	42
6	*Chrysanthemum adustum*	Albula	Fl	89	92	9	19	377	447	269	336	79	88
7	*Crepis aurea*	Albula	Fl	136	141	37	29	324	319	235	253	89	42
8	*Plantago atrata*	Albula	Fl	117	103	9	14	463	425	316	311	114	76
9	*Rhinanthus alectorolophus*	Albula	Fl	151	135	17	26	291	322	206	236	133	65
10	*Rumex arifolius*	Albula	Fr	121	123	17	14	387	430	251	301	53	48
	Leguminous herbs												
11	*Anthyllis vulneraria*	Albula	Fl	129	132	11	16	365	378	270	261	75	67
12	*Hedysarum hedysaroides*	Albula	Fl	207	239	17	19	317	320	201	212	63	62
13	*Trifolium badium*	Albula	Fl	139	152	11	17	315	326	265	266	82	81
	Trees												
14	*Castanea sativa* (leaves)	Misox	–	129	153	22	16	359	444	240	302	76	103
15	*Fraxinus excelsior* (leaves)	Misox	–	179	141	17	11	442	374	381	284	182	80
16	*Sambucus nigra* (leaves)	Sufers	–	234	251	33	36	245	190	155	159	52	48
17	*Sambucus nigra* (flowers)	Sufers	–	257	237	35	28	263	256	212	217	134	72
	SEM			11.4	11.2	2.3	1.7	29.1	31.9	14.2	11.9	9.2	4.6

ADF, acid detergent fibre; ADL, acid detergent lignin; Bu, budding; Fl, flowering; Fr, fruiting; NDF, neutral detergent fibre; SEM, standard error of the mean.

plants included two grasses, eight non-leguminous herbs and three herbaceous legumes. These plants were cut at 1 cm above ground. Additionally, leaves or flowers of three tree species were included because they were commonly fed to domestic ruminants in the alpine region in former times[18] and were supposed to contain high levels of phenolic compounds. The plants were classified into four functional groups, i.e. grasses, non-leguminous herbs, leguminous herbs and trees. Approximately 0.5 kg of fresh matter was collected from each plant species in both years. All plants in both years were sampled from at least five plots within areas of at least 1 ha at the respective sites as indicated in Table 1. After cutting, plants were pooled into one sample per species. 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.

Analyses of the chemical composition

The dried and milled samples were analysed for their chemical composition in 2009 and 2010 shortly after collection. The proximate contents were determined using standard procedures.[19] Determinations included dry matter (DM) and total ash (TA) using a TGA-500 furnace (Leco Corporation, St Joseph, MI, USA; AOAC index no. 942.05), crude protein (CP) using a C/N analyser (Leco-Analysator Typ FP-2000; Leco Instrumente GmbH, Kirchheim, Germany; AOAC index no. 977.02), and ether extract (EE) using a Soxhlet extractor (Extraktionssystem B-811; Büchi, Flawil, Switzerland; AOAC index no. 963.15). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Van Soest *et al.*[20] using the Fibertec apparatus (Fibertec System M, 1020 Hot Extraction; Tecator, Flawil, Switzerland). In the case of NDF, α-amylase was added but no sodium sulfite treatment was applied. Detergent fibre values were expressed excluding residual ash. Subtraction of CP, EE, NDF and total phenols from organic matter (OM) gave the non-fibre carbohydrates (NFC).

Determinations of phenolic fractions, i.e. total phenols (TP), total tannins (TT) and condensed tannins (CT) in the samples were carried out based on Makkar.[21] In doing so, TP and TT were assessed by a modified Folin–Ciocalteu method using polyvinylpolypyrrolidone to separate non-tannin phenols (NTP) from tannin phenols. The CT were analysed by the butanol–HCl–iron method. While both TP and TT were expressed as gallic acid equivalents, CT was given as leucocyanidin equivalents. Hydrolysable tannins (HT) were calculated as the difference between TT and CT.[22] Analyses were carried out in duplicate except for detergent fractions which were measured in triplicate.

In vitro incubation

Incubation with rumen fluid was carried out with the Hohenheim Gas Test (HGT) apparatus according to the protocol of Menke and Steingass[23] using modified syringes.[24] The modified syringe has two outlets. One outlet is designed for filling and emptying the liquid phase and the other outlet, which is covered with a polytetrafluoroethylene layer, is designed for sampling from the gas phase. The *in vitro* incubation was conducted for the harvests of 2009 and 2010 in order to observe the consistency of plant species in their chemical composition and the effects on *in vitro* rumen fermentation across the two years. The samples were incubated for 24 h at 39 °C in four replicates (runs) each, represented by one syringe per replicate. In each syringe, 200 mg DM of plant sample was incubated with 10 mL of ruminal fluid

and 20 mL of HGT buffer solution.[23] Rumen fluid was obtained from a rumen-fistulated Brown Swiss cow, which was cared for according to Swiss guidelines for animal welfare and received white clover–ryegrass hay (*ad libitum*) and 0.5 kg day^{-1} of a regular dairy concentrate (UFA 149; UFA AG, Herzogenbuchsee, Switzerland). Before incubation, rumen fluid was strained through gauze (four layers; 1 mm pore size, Type 17 MedPro; Novamed AG, Flawil, Switzerland). In additional, syringes without feed (blanks) or with standard concentrate or hay (obtained from the Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany) were incubated in triplicate in each run. These incubations served as controls for successful incubation and as adjustment factors to estimate *in vitro* organic matter digestibility (IVOMD) and content of metabolisable energy (ME).[23]

After 24 h of incubation, the volume of fermentation gas produced was read from the calibrated scale on the glass syringes. The incubation was terminated by decanting the liquid phase from all syringes from the first outlet while the fermentation gas was left inside. Subsequently, 0.15 mL of the fermentation gas was obtained through the second, covered, outlet using a small gas-collection syringe (Hamilton AG, Bonaduz, Switzerland) through a gas-tight septum covering the second outlet of the incubation syringes. These samples were analysed on a Hewlett Packard gas chromatograph (model 5890 Series II; Hewlett Packard, Avondale, PA, USA) for CH_4 concentration.[24]

In the fermentation fluid, pH and ammonia were analysed with a potentiometer (model 632; Metrohm, Herisau, Switzerland) equipped with respective electrodes. Protozoal and bacterial counts in fermentation fluid were determined using Bürker counting chambers (0.1 mm and 0.02 mm depth, respectively; Blau Brand, Wertheim, Germany). Samples were fixed with Hayem solution (mg mL^{-1}: $HgCl_2$, 2.5; Na_2SO_4, 25; NaCl, 5.0) for bacterial counting and with 1 : 10 diluted formalin (40 w/v of water) for protozoal counting. Short-chain fatty acid (SCFA) analyses in fermentation fluid were determined[25] using a HPLC (LaChrom, L-7000 series; Hitachi Ltd, Tokyo, Japan) to separate C_2 (acetate), C_3 (propionate), C_4 (butyrate), isoC_4, C_5 (valerate) and isoC_5.

Following the standard equations,[23] the following variables were calculated from gas production (GP; actual values adjusted by the values recorded with Hohenheim standard hay and concentrate) and proximate contents:

$$\text{IVOMD} = 148.8 + 8.893\text{GP} + 0.448\text{CP} + 0.651\text{TA}$$

and

$$\text{ME} = 3.16 + 0.0695\text{GP} + 0.000\,730\text{GP}^2 + 0.00\,732\text{CP} + 0.02\,052\text{EE}$$

where the units are: IVOMD, mg g^{-1}; GP, mL; CP, g kg^{-1} DM; TA, g kg^{-1} DM; ME, MJ kg^{-1}; and EE, g kg^{-1} DM.

Statistical analysis

Data were subjected to analysis of variance using PROC MIXED of SAS.[26] This was applied to the fermentation data in each harvest year (2009 and 2010) by considering incubation run ($n = 4$) as a random variable. The fixed effect that was tested was plant species ($n = 17$). The LSMEANS statement was performed as the basis for multiple comparisons among means using the Tukey–Kramer method.[26] Data of 2009 and 2010 were compared using the *t*-test. Principal component analysis (PCA) was performed on the data (average of both years) using SPSS statistical software version 17.0.[27] Data included in PCA were chemical composition and *in vitro* rumen fermentation variables. Data from *C. sativa* were excluded from the PCA since this species contained an extremely high HT content, and produced a very low CH_4/total gas which may therefore suggest a closer relationship than is actually present. Extraction of the principal components (PCs) was based on eigenvalues greater than 1 with the varimax rotation method. However, only the first two PCs were displayed and used for calculating the factor scores of each plant.

RESULTS

Chemical composition of the plants

Consistently, in both harvest years (2009 and 2010) CP contents in *Sambucus nigra* flowers and leaves were found to be among the highest across the experimental plants, followed by *Hedysarum hedysaroides* (Table 1). The lowest CP concentrations occurred in plants from the non-leguminous herbs (*Chrysanthemum adustum* and *Plantago atrata*), also in both years. Apart from *Crepis aurea* and *S. nigra*, the plants were relatively poor in EE (mostly <25 g kg^{-1} DM). The NDF contents were especially high in the two grasses, *Nardus stricta* and *Poa alpina*, and lowest in the parts of *S. nigra*. Variation in the other fibre fractions was considerable but not systematic across the functional groups. The concentrations of the phenolic fractions varied across all functional groups (Table 2). *Alchemilla xanthochlora, H. hedysaroides* and *Castanea sativa* contained >50 g TP kg^{-1} DM in 2009 and 2010. Hydrolysable tannins represented the largest part of the phenols in *A. xanthochlora* and *C. sativa*. No plant contained substantial amounts of condensed tannins except *H. hedysaroides* harvested in 2010.

Ruminal fermentation and methanogenesis

Incubation of hay and concentrate standards resulted in CH_4/total gas values of 161 $\pm$ 25.3 and 182 $\pm$ 25.1 mL L^{-1}, respectively (measured for 2010 only). The ruminal fermentation traits of the grasses did not differ generally from those in the other functional groups. *N. stricta* resulted in a significantly lower concentration of total SCFA than *P. alpina* ($P < 0.05$) after 24 h of incubation in the fermentation fluid (Table 3). The proportion of all individual SCFA (not shown for C_4, isoC_4, C_5 and isoC_5) did not differ between the two species. Likewise, the NH_3 concentration in the fermentation fluid was not different, either. In terms of nutritional quality, *P. alpina* was superior to *N. stricta* due to higher values of total gas produced, IVOMD and ME content ($P < 0.05$), and this was consistently observed in 2009 and 2010 (Table 4). Further, *P. alpina* produced lower CH_4/total gas compared to *N. stricta* ($P < 0.05$) in 2009 although the effect was not significant in 2010. However, the difference was not significant between the two grasses when expressed as CH_4/organic matter digestible *in vitro*.

The NH_3 concentration varied considerably in the group of non-leguminous herbs ($P < 0.05$), while total SCFA did not differ significantly across plants within the group in both years (2009 and 2010). Total gas production was highest when incubating *C. aurea* (consistently in 2009 and 2010), followed by *A. xanthochlora* (in 2009) or *Rhinanthus alectorolophus* (in 2010). A closely similar pattern was also obtained for IVOMD. *Crepis aurea, A. xanthochlora* and *R. alectorolophus* contained superior ME values in the non-leguminous herbs group in both years. No difference occurred for CH_4/total gas across all non-leguminous herbs.

No significant difference was found between leguminous herbs in terms of NH_3 and SCFA profiles in 2009 or 2010. Incubation of

Table 2. Phenolic contents of the experimental plants collected in 2009 and 2010 (g kg^{-1} dry matter; $n = 2$ per year)

		Total phenols[a]		Non-tannin phenols[a]		Total tannins[a]		Condensed tannins[b]		Hydrolysable tannins[c]	
No.	Plant species	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
	Grasses										
1	*Nardus stricta*	7	9	7	9	ND	ND	ND	ND	ND	ND
2	*Poa alpina*	18	20	14	15	4	5	ND	1	4	4
	Non-leguminous herbs										
3	*Achillea millefolium*	12	34	9	29	3	5	ND	1	3	4
4	*Alchemilla xanthochlora*	55	52	21	15	34	37	1	2	33	35
5	*Carum carvi*	18	23	12	17	6	6	ND	ND	6	6
6	*Chrysanthemum adustum*	17	23	12	16	5	7	ND	ND	5	7
7	*Crepis aurea*	19	29	14	20	5	9	1	ND	4	9
8	*Plantago atrata*	21	30	13	23	8	7	ND	1	8	6
9	*Rhinanthus alectorolophus*	29	29	19	21	10	8	1	1	9	7
10	*Rumex arifolius*	33	43	15	17	18	26	5	13	13	13
	Leguminous herbs										
11	*Anthyllis vulneraria*	26	23	16	18	10	5	2	2	8	3
12	*Hedysarum hedysaroides*	67	70	32	30	35	40	9	32	26	8
13	*Trifolium badium*	41	45	21	23	20	22	5	10	15	12
	Trees										
14	*Castanea sativa* (leaves)	92	131	13	9	79	122	1	2	78	119
15	*Fraxinus excelsior* (leaves)	13	30	12	25	1	5	ND	ND	1	5
16	*Sambucus nigra* (leaves)	22	50	22	47	ND	3	ND	ND	ND	3
17	*Sambucus nigra* (flowers)	40	44	28	33	12	11	1	1	11	10
	SEM	5.4	6.7	1.6	2.3	4.8	7.1	0.6	2.0	4.6	6.8

ND, not detected; SEM, standard error of the mean.
[a] As gallic acid equivalents.
[b] As leucocyanidin equivalents.
[c] Obtained by difference between total tannins and condensed tannins.

H. hedysaroides resulted in significantly lower total gas production and IVOMD than the other two plants within this functional group ($P < 0.05$) which resulted in a significantly lower ME value in 2010. Further, with *H. hedysaroides* the CH_4 production per IVOMD was lower in 2010 than in 2009. Despite this, CH_4/total gas and CH_4/digestible organic matter was similar across all three leguminous herbs in both years.

Corresponding to the high CP contents, ruminal NH_3 concentrations were higher after incubation of both *S. nigra* flowers and leaves compared to all other species across the different functional groups ($P < 0.05$), consistently in 2009 and 2010. Within tree species, lower total SCFA with a higher proportion of C_3 ($P < 0.05$) were observed in the *C. sativa* incubation. This plant produced extremely low total gas, CH_4/total gas and CH_4/digestible organic matter as well as IVOMD and ME values compared to all other plants ($P < 0.05$). These patterns were consistently observed in 2009 and 2010. Bacterial and protozoal counts did not significantly differ across plant species.

Principal component analysis

The first two principal components (PC1 and PC2) explained 33.8% and 19.9% of the total variation, respectively (Fig. 1). The position of each variable in the loading plot (Fig. 1a) describes its relationship to other variables. Total SCFA and IVOMD were positively related with each other and with NFC, but negatively related to NDF and ADF contents in the plants. By contrast, within the dataset, ADL appeared not to be related with any other variable. CH_4/total gas was in inverse direction to TP, TT, HT and NFC but in the same direction with NDF and ADF. The content of CP was positively related to ruminal bacteria and NH_3 concentration.

The individual plants were classified based on the loading plot result, combined with the factor score of each plant (Fig. 1b). Most of the plants were centred in the origin of the plot and only a few plants had some distinct features. The cluster in the origin did not show any differentiation concerning the functional groups. *Nardus stricta* appeared clearly outside the cluster and was associated with high NDF and ADF, and high CH_4/total gas. This implied that the plant had relatively low total SCFA production and IVOMD. By contrast, *A. xanthochlora* was outside the cluster in the inverse direction to that of *N. stricta* and may be considered as a good-quality plant based on the PCA result. Both *S. nigra* flowers and leaves were in the same direction with CP and NH_3 variables.

DISCUSSION

Despite the increasing scientific interest in mountain pastures as feed resources,[28] the phenolic compounds and potential anti-methanogenic properties of individual alpine forage plants have not yet been comprehensively studied. It was the objective of the current study to provide indications on these properties by investigating 17 alpine plant species from different functional groups. In contrast to our expectations, the overall variation in nutrient composition and the resulting fermentation properties was low. With one exception, no plant represented considerably high concentrations of phenols, and the methane mitigation

Table 3. Ruminal fermentation characteristics of the experimental plants ($n = 4$ per year)

	NH_3 (mmol L^{-1})		SCFA (mmol L^{-1})		C_2 (% of total)		C_3 (% of total)		Bacteria (10^9 mL^{-1})		Protozoa (10^4 mL^{-1})	
Plant species	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Grasses												
Nardus stricta	9.1^{abc}	12.1^{c}	68.2^{b}	69.8^{ab}	70.8^{ab}	75.7^{ab}	19.8^{ab}	14.1^{abc*}	3.31	2.69	2.78	2.22
Poa alpina	7.7^{abc}	12.0^{c}	85.9^{e}	90.0^{b}	68.1^{ab}	77.2^{abc*}	22.0^{ab}	13.4^{abc*}	2.83	2.39	3.16	2.50
Non-leguminous herbs												
Achillea millefolium	9.5^{bc}	12.6^{c}	82.8^{cde}	91.2^{b*}	74.2^{b}	83.6^{c*}	17.9^{ab}	9.9^{ab*}	3.25	3.00	2.50	2.45
Alchemilla xanthochlora	8.0^{abc}	8.6^{ab}	88.6^{e}	101.4^{b}	73.6^{b}	82.7^{bc*}	17.6^{ab}	9.7^{a*}	2.73	2.79	4.56	2.95*
Carum carvi	9.0^{abc}	12.7^{c}	87.8^{e}	95.8^{b}	72.4^{ab}	81.0^{bc*}	19.7^{ab}	11.8^{abc*}	3.73	2.94	3.39	2.67
Chrysanthemum adustum	6.3^{a}	11.4^{bc*}	86.7^{e}	90.7^{b}	72.9^{b}	82.1^{bc*}	19.1^{ab}	10.1^{ab*}	2.64	2.52	3.06	2.45
Crepis aurea	10.3^{cd}	11.8^{c}	89.6^{e}	105.6^{b*}	72.4^{ab}	81.9^{bc*}	18.9^{ab}	10.5^{ab*}	3.14	2.87	3.66	2.05
Plantago atrata	6.3^{a}	8.1^{a}	79.5^{bcde}	74.8^{ab}	70.9^{ab}	79.4^{abc}	20.5^{ab}	11.6^{abc*}	2.15	2.69	4.06	2.23
Rhinanthus alectorolophus	7.7^{abc}	9.8^{abc}	86.9^{e}	98.8^{b}	70.9^{ab}	79.5^{abc*}	20.4^{ab}	11.7^{abc*}	2.65	3.08	3.83	2.89
Rumex arifolius	6.5^{ab}	10.0^{abc}	83.9^{de}	88.3^{b}	72.0^{ab}	77.3^{abc}	19.1^{ab}	13.9^{abc}	3.92	2.77	4.12	2.00
Leguminous herbs												
Anthyllis vulneraria	6.5^{ab}	10.6^{abc}	81.5^{cde}	104.9^{b}	74.2^{b}	79.0^{abc}	18.4^{ab}	13.3^{abc}	3.77	3.06	2.17	2.72
Hedysarum hedysaroides	7.3^{abc}	10.1^{abc}	71.2^{bcd}	82.9^{b}	73.4^{b}	77.9^{abc}	19.3^{ab}	14.6^{bc}	3.56	2.82	2.73	2.67
Trifolium badium	6.5^{ab}	10.2^{abc}	82.3^{cde}	94.6^{b}	71.7^{ab}	79.0^{abc}	19.6^{ab}	13.4^{abc*}	3.79	3.19	2.89	3.33
Trees												
Castanea sativa (leaves)	6.9^{ab}	8.1^{a}	46.4^{a}	41.7^{a}	66.2^{a}	72.3^{a}	22.9^{b}	16.3^{c*}	3.37	3.67	2.89	3.63
Fraxinus excelsior (leaves)	7.1^{ab}	8.0^{a}	70.1^{bc}	73.4^{ab}	73.6^{b}	78.0^{abc}	17.1^{a}	13.8^{abc}	3.14	3.06	3.83	2.45
Sambucus nigra (leaves)	12.8^{d}	16.3^{d}	83.6^{de}	87.9^{b}	73.3^{b}	78.3^{abc}	19.2^{ab}	12.8^{abc*}	3.08	4.25	3.06	2.11
Sambucus nigra (flowers)	16.0^{e}	17.0^{d}	91.5^{e}	99.0^{b}	73.9^{b}	81.2^{bc}	18.4^{ab}	11.4^{ab*}	3.75	3.75	3.55	2.50
SEM	0.36	0.49	1.44	3.11	0.38	0.63	0.30	0.40	0.115	0.113	0.242	0.085
P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.033	<0.001	NS	NS	NS	NS

C_2, acetate; C_3, propionate; NS, not significant; SCFA, short-chain fatty acid, SEM, standard error of the mean.
[a–e] Mean values in the same column without common superscript are significantly different at $P < 0.05$.
* Significantly different at $P < 0.05$ between 2009 and 2010 within the same plant species.

potential was similarly limited. However, several yet rarely considered plants proved to have a high nutritive value and to represent an interesting forage resource for ruminants.

Alpine grasses

The two grasses investigated were typical for alpine pastures and represent either a good (*Poa alpina*) or a poor (*Nardus stricta*) forage[11,13] as can be concluded from the higher NDF and lower ME contents of *N. stricta* compared to *P. alpina*. *Nardus stricta* is generally known as a rather unpalatable plant species, containing high amounts of poorly digestible cell wall constituents, and it is largely avoided by domestic grazers.[12,29,30] This corresponds with findings on *in vivo* digestibility of alpine swards dominated by *N. stricta*.[31] *Poa alpina* was superior to *N. stricta* also in terms of a lower CH_4 emission potential at a given gas production (i.e. digestibility and energy supply). This might be explained by its lower NDF and ADF contents, and its higher proportion of the non-fibre fraction compared to *N. stricta*. Dietary cell-wall content and ruminal CH_4 production are considered to be positively correlated since fibre degradation results in large amounts of hydrogen which is utilised by the methanogens for CH_4 formation.[32] Although TP may be negatively related to CH_4 formation (see the PCA result) and TP contents were higher in *P. alpina* than in *N. stricta*, the TP concentration has to be considered to be too low to become the determining factor for the observation made.[16] It cannot be excluded that phenol degradation occurred during sample conservation. However, the drying temperature of 60 °C did not lead to a general decrease in the extractability of tannins nor to adverse effects on the biological activity of tannins compared to freeze-dried and freshly frozen samples in own unpublished tests and in a study of Muetzel and Becker.[33]

Alpine herbs

When compared to other studies on alpine forage herbs at similar vegetation stages,[3,11,13] most of the investigated herbs (leguminous and non-leguminous) showed comparable nutrient concentrations and fermentation properties, resulting in satisfactory IVOMD and energy contents. Nevertheless, some plant species had unexpectedly low IVOMD and ME values, particularly *Plantago atrata* and *Anthyllis vulneraria*, which have been described elsewhere as valuable forage plants for dairy cows.[3,11,17] This could have been related to seasonal effects, which are not covered by the current study; however, an improvement of the nutritional value in later season is not to be expected.[3] The comparably high ME content of *C. aurea*, as estimated in the present study, was also observed by Schubiger *et al.*[11] The comparably low NH_3 values in the incubations of the high-CP plant *Hedysarium hedysaroides* might have been the result of the influence of the condensed tannins.[34]

In contrast to expectations based on larger differences among herbaceous species,[35] the actual variation in CH_4/total gas production was low within the herbaceous species, as was the variability in TP contents compared to other studies.[15] The lowest CH_4/total gas ratio within the herbs, measured when incubating *A. xanthochlora*, especially in 2009, might be related to the relatively high content of phenolic compounds, particularly HT.

Table 4. Gas and CH_4 production from, and *in vitro* organic matter (OM) digestibility (IVOMD) as well as metabolisable energy (ME) of, the experimental plants ($n = 4$ per year)

	Total gas (mL)		IVOMD (mg g^{-1})		ME (MJ kg^{-1})		CH_4/total gas (mL L^{-1})		CH_4/OM digestible *in vitro* (mL g^{-1})	
Plant species	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Grasses										
Nardus stricta	28.1^{b}	33.1^{bc}	500^{b}	550^{b}	6.89^{b}	7.70^{b}	200^{e}	153^{b}	60.2^{f}	46.2^{b}
Poa alpina	44.4^{ghi}	48.8^{g}	667^{ef}	728^{ef}	9.47^{fg}	10.52^{gh}*	148^{bcd}	127^{b}	52.0^{def}	42.8^{b}
Non-leguminous herbs										
Achillea millefolium	34.1^{cd}	42.6^{def}*	664^{ef}	717^{def}*	8.59^{cde}	9.61^{ef}*	157^{bcd}	123^{b}*	43.2^{bcd}	36.8^{b}
Alchemilla xanthochlora	49.8^{ij}	46.1^{fg}	753^{g}	719^{def}	10.58^{hi}	10.11^{fgh}	131^{bc}	137^{b}	45.5^{bcde}	44.2^{b}
Carum carvi	44.3^{ghi}	44.2^{efg}	702^{fg}	716^{def}	9.42^{fg}	9.72^{ef}	158^{bcd}	147^{b}	52.3^{def}	45.2^{b}
Chrysanthemum adustum	44.6^{hi}	40.3^{de}*	663^{ef}	632^{c}*	9.05^{def}	8.78^{cd}	151^{bcd}	131^{b}	53.5^{ef}	42.1^{b}
Crepis aurea	51.1^{j}	48.6^{g}	748^{g}	741^{f}	10.97^{i}	10.64^{h}	149^{bcd}	147^{b}	53.6^{ef}	48.5^{b}
Plantago atrata	37.8^{def}	39.7^{de}	602^{cd}	639^{c}	8.26^{cd}	8.66^{cd}	163^{cd}	148^{b}	54.3^{ef}	46.7^{b}
Rhinanthus alectorolophus	46.9^{hij}	46.8^{fg}	736^{g}	713^{def}	9.99^{gh}	10.26^{fgh}	142^{bcd}	150^{b}	47.6^{bcde}	49.3^{b}
Rumex arifolius	42.3^{efgh}	38.0^{cd}	653^{def}	621^{c}	9.09^{ef}	8.56^{cd}	149^{bcd}	134^{b}	50.9^{cdef}	41.0^{b}
Leguminous herbs										
Anthyllis vulneraria	38.8^{defg}	42.1^{def}*	645de	703^{def}*	8.53^{cde}	9.29^{de}*	167de	154^{b}	53.2^{ef}	46.1^{b}
Hedysarum hedysaroides	30.9^{bc}	28.2^{b}	571^{c}	565^{b}	8.17^{c}	8.18^{bc}	164^{cd}	156^{b}	47.6^{bcde}	38.9^{b}*
Trifolium badium	36.9^{de}	40.7^{de}*	631de	669^{cd}*	8.33^{cde}	9.24^{de}*	157^{bcd}	148^{b}	48.9^{bcde}	45.3^{b}
Trees										
Castanea sativa (leaves)	10.4^{a}	2.7^{a}*	340^{a}	271^{a}*	5.43^{a}	4.83^{a}*	7^{a}	42^{a}	1.2^{a}	2.4^{a}
Fraxinus excelsior (leaves)	38.9^{defg}	30.0^{b}*	654^{def}	559^{b}*	9.03^{def}	7.52^{b}*	156^{bcd}	144^{b}	49.3^{cde}	38.9^{b}
Sambucus nigra (leaves)	41.4^{efgh}	37.8^{cd}*	728^{g}	696^{def}*	10.12^{gh}	9.93^{efgh}	138^{bcd}	146^{b}	41.5^{bc}	40.0^{b}
Sambucus nigra (flowers)	43.1^{fgh}	39.0^{d}*	734^{g}	690de*	10.59^{hi}	9.83^{efg}*	127^{b}	131^{b}	39.5^{b}	36.9^{b}
SEM	1.19	1.32	1.27	1.38	0.171	0.175	5.1	4.1	1.67	1.58
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of the mean.
[a–j] Mean values in the same column without common superscript are significantly different at $P < 0.05$.
* Significantly different at $P < 0.05$ between 2009 and 2010 within the same plant species.

This was supported by the results of the PCA. The CH_4-mitigating properties of HT have been previously demonstrated.[36] However, it should be noted that *A. xanthochlora* contains several classes of essential oils[37] which also may influence CH_4 production in the rumen.[38] In addition, *H. hedysaroides* contained relatively high amounts of TP but with this plant, total gas production rather than the relative proportion of CH_4 was reduced. The reduction in total gas production from *Achillea millefolium* cannot be explained with TP, which were unexpectedly low for this species,[15,39] but rather with the unfavourable proportion of NFC (data not shown) or by the essential oils[40] which were not measured here. *Hedysarum hedysaroides, A. millefolium* and *A. xanthochlora* showed a similar tendency toward low CH_4 production per unit of IVOMD, but the energetic value of *A. xanthochlora* was higher than that of the former two plants. This could be explained by the lower content of NDF, thus demonstrating that *Alchemilla* spp. represent a particularly valuable forage for ruminants in the alpine region, as also indicated in applied[17] and scientific literature.[13] The indication for a reduced CH_4 emission when fermenting *A. millefolium* and *H. hedysaroides*, however, needs further confirmation, taking into account that the phenolic contents of mountain forages may vary during the vegetative development of the plants.[15]

Alpine trees

Among the tree samples, *S. nigra* flowers and leaves proved to be forages with a good nutritional quality fitting into the range of the herbs. For *Fraxinus excelsior*, the nutritive quality was not consistently as high as reported for other *Fraxinus* spp.[41] This tree genus was commonly used as a forage source in European alpine regions until the middle of the 20th century[18] and in Mediterranean region it is still in use.[41] However, neither of these two tree species substantially lowered the CH_4 production per unit of digestible OM, as compared with the herbs.

Although, previously, *C. sativa* leaves have been commonly fed to ruminants[18] and are browsed by deer,[42] the very low total gas production and low values of IVOMD and ME found in the present analysis indicate a limited forage quality. Due to the limited feeding value, it might be not possible to profit from the low CH_4/total gas ratio resulting from *C. sativa* incubations by feeding these leaves at high proportions. However, this does not exclude that these leaves could moderately mitigate methane production when fed only in low proportions. *Castanea sativa* leaves contained by far the most HT of all plants investigated. Effects of castanea extracts on ruminal CH_4 emissions have been reported previously.[36,43] Increasing dietary concentrations of chestnut HT from 132 to 185 g HT kg^{-1} DM decreased CH_4/total gas from 134 to 88 mL L^{-1}, respectively,[36] but this decrease was not accompanied by reductions of methanogen and protozoal populations. In the present study, the HT concentration in the *C. sativa* leaves was lower than the dietary HT concentrations used by Bhatta *et al.*[36] but the CH_4 reduction effect was much higher, suggesting that not only the HT fraction affects CH_4 production when whole leaves instead of extracts[36,43] are used.

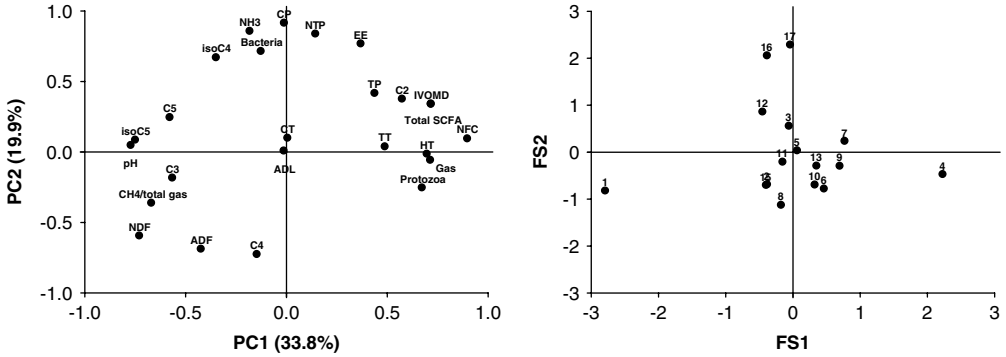

Figure 1. Plot of the first two PC loadings, describing the relationship among variables of plant composition and *in vitro* ruminal fermentation (a), and plot of the first two factor scores, describing the classification of each plant within the PC loading (b) (number codes for plants are explained in Table 1). ADF, acid detergent fibre; ADL, acid detergent lignin; C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; CP, crude protein; CT, condensed tannins; EE, ether extract; FS, factor score; HT, hydrolysable tannins; IVOMD, *in vitro* organic matter digestibility; NDF, neutral detergent fibre; NFC, non-fibre carbohydrates; NTP, non-tannin phenols; PC, principal component; SCFA, short-chain fatty acid; TP, total phenols; TT, total tannins.

CONCLUSIONS

The nutritive value of the alpine forage plants investigated was often high. This is especially true for *Alchemilla xanthochlora* and *Crepis aurea* among the herbaceous plants and *Sambucus nigra* among the trees. The *in vitro* ruminal CH_4 emission potential and the concentration of phenolic compounds of the Alpine forage plants varied less than expected. Data did not support the hypothesis of this study that alpine swards contain plants with particularly high amounts of phenolic compounds, leading to an overall low CH_4-emission potential of alpine grazing systems. Nevertheless, there were some interesting plants in this respect (*Achillea millefolium, Alchemilla xanthochlora, Sambucus nigra*). *Castanea sativa*, being a very effective CH_4 inhibitor, also had the poorest ruminal fermentation rates. Whether the changing plant composition in the later season modifies fermentation properties has to be subject of further research.

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