

METHANE EMISSION BY ALPACA AND SHEEP FED ON FORAGES

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ABSTRACT

Six male alpaca and six castrated Romney sheep were simultaneously and successively fed on lucerne hay (Experiment 1), grazed ryegrass/white clover pasture (RG/WC) (Experiment 2) and grazed birdsfoot trefoil pasture (Lotus) (Experiment 3). CH₄ emission, voluntary feed intake (VFI), diet quality and protozoa count in forestomach contents were determined. Diets selected by alpaca were of lower quality than those selected by sheep, and the VFI of dry matter (DMI, g kg⁻¹ LW^{0.75}) were consistently lower ($P<0.001$) for the alpaca than for the sheep (38.8 v. 74.0, 33.5 v. 70.0 and 40.3 v. 128.0 on lucerne hay, RG/WC and Lotus, respectively). Alpaca and sheep did not differ ($P>0.05$) in their CH₄ emissions (% gross energy intake, %GEI) when fed on lucerne hay (5.1 v. 4.7), but they did differ when fed on RG/WC (9.4 v. 7.5, $P<0.04$) and Lotus (6.4 v. 2.7, $P<0.001$). In addition to the depressed CH₄ emission (%GEI) by sheep grazing Lotus, protozoa populations in these sheep increased four-fold in comparison to the other forages. On lucerne hay, alpaca had higher ($P<0.05$) NDF digestibilities (47.8 v. 46.1%) than sheep. Differences between animal species in: (1) chemical composition of their diets (more fibrous in alpaca), (2) VFI (lower in alpaca), and (3) digestibility of cell walls (higher in alpaca), are consistent with alpaca having a lower fractional outflow of feed particles from their forestomach than the sheep. Thus, differences between these species in outflow of feed particles from their forestomach might have been the underlying physiological mechanism responsible for the differences in CH₄ emission (%GEI).

1.0 INTRODUCTION

Our previous study (Pinares-Patiño et al., 2001) under controlled conditions showed that rumen particulate fractional outflow rate (particulate FOR) was a major contributor to the differences between sheep in methane (CH₄) emission. Sheep with lower particulate FOR had larger rumen fills and higher feed digestibilities and CH₄ emissions. Direct measurement of the particulate FOR and rumen fill is difficult under grazing conditions. Then, the study of CH₄ emission rates by species or breeds differing in these animal factors might yield further insights into their involvement in CH₄ emission.

South American camelids (SAC) differ from sheep in the structure and function of their digestive system and therefore in their nutritional strategies, being the former species more efficient in digesting plant cell walls than sheep (San Martin, 1987). This higher digestibility was attributed to a lower particulate FOR (San Martin, 1987; Lemosquet et al., 1996). Based on the above knowledge, this study tested the hypothesis that alpaca and sheep would differ in their CH₄ emissions when fed on three different forages.

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

The study was carried out from October to December 1999 and involved three successive experiments (1, 2 and 3), during which 6 male alpaca (Huacaya, 18-month old and 61.4 ± 10.5 kg LW) and 6 castrated sheep (Romney, 15-month old and 43.0 ± 1.8 kg LW) were fed *ad libitum* (once a day feeding) on chaffed lucerne (*Medicago sativa*) (Experiment 1), or grazed under generous pasture allowances on ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture (hereafter named 'RG/WC') (Experiment 2) or birdsfoot trefoil (*Lotus corniculatus*) pasture (hereafter named 'Lotus') (Experiment 3). All 12 animals were fed on the same forage at the same time.

During grazing experiments alpaca and sheep were grazed on paired plots as separate flocks. Within each plot, a fresh strip of pasture was grazed each day. Daily herbage allowance was controlled by electric fences to offer 12% of their body weight in total herbage dry matter (DM). RG/WC pasture was at flowering stage whereas Lotus was vegetative. Herbage mass of RG/WC was $3500 \text{ kg DM ha}^{-1}$, whereas that of Lotus was $5700 \text{ kg DM ha}^{-1}$. Lotus herbage had high proportion (53%) of stems and because of that daily pasture allowance was on leaf DM basis.

Experiment 1 (indoors) was 29 days in duration, whereas grazing experiments were 21 days in duration. At each experiment, animals were acclimatized for 15 days. Measurements in Experiment 1 included: voluntary feed intake (VFI) (d 16–21), CH_4 emission and feed digestibility (d 22–27) and protozoa counts (d 28–29), whereas in Experiments 2 and 3, CH_4 emission, faecal output and diet quality were measured over d 16–19 and protozoa counts over d 20–21.

2.2 SAMPLE COLLECTION

During Experiment 1, both feed on offer and faecal outputs were recorded daily. Samples were taken for both DM determination (100°C , 48 h) and chemical analysis on frozen-stored (-20°C) samples. The amounts of feed refused were recorded daily and samples taken for daily DM determination. Other samples of daily feed refusals were stored frozen for chemical analyses. After the collection, all frozen samples were pooled within animals, mixed thoroughly and re-sampled, then freeze-dried, ground and used for analysis.

Daily CH_4 emission was measured by the sulphur hexafluoride (SF_6) tracer technique (Johnson et al., 1994) as described by Pinares-Patiño et al. (2001) (Experiment 1) or by Lassey et al. (1997) (Experiments 2 and 3).

Rumen contents (15–20 ml) were sampled using a stomach tube. Samplings took place between 2.5 to 3.0 h post feeding (Experiment 1) or 1 h after the morning grazing bout (Experiments 2 and 3). Samples for protozoa counting were prepared as described by Pinares-Patiño et al. (2001).

Samples of pasture on offer were obtained daily before animals entered the allocated pasture strips. Four 0.10 m² quadrates (0.40×0.25m) were cut at ground level, weighed, pooled, and subsampled for DM determination. Other daily samples of the pooled material were stored frozen for later within-period pooling, freeze drying, grinding and chemical analyses.

For each animal species, samples of the herbage which was grazed (diet) were collected from within three 0.5 m² protected (using 1.0×0.5 m wire cages) areas. The samples were taken to simulate the diet selected by the animals grazing that strip. Daily samples were stored frozen and later pooled within animal species, freeze dried, ground and used for chemical analyses.

Total faeces outputs by the grazing animals were collected twice-daily using a harness and canvas bag. Faeces from each animal were weighed, pooled within each day and sampled for DM determination. Other subsamples (10%) of the daily faeces output were stored frozen and later pooled within animal species, sub-sampled, freeze dried, ground and used for chemical analysis.

At grazing, daily DMI by each individual alpaca and sheep were estimated from the *in vitro* pasture DM digestibilities (DMD) in conjunction with the total faecal DM output by the individual animals.

2.3 ANALYTICAL METHODS

Organic matter (OM), gross energy (GE), nitrogen (N), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents and protozoa numbers were determined as described by Pinares-Patiño et al. (2001).

Samples of herbage eaten underwent *in vitro* OM digestibility (*in vitro* OMD) determinations by the enzymatic method of Roughan and Holland (1977). Samples of the pasture (RG/WC and Lotus) on offer and eaten were analysed for extractable and bound condensed tannins (CT) (Terrill et al., 1992).

Data for CH₄, VFI, and protozoa counts were analysed within each experiment (or forage) using Proc GLM of SAS Institute, Inc. Data for apparent *in vivo* feed digestibility measured in Experiment 1, were also subjected to analysis of variance.

3.0 RESULTS

3.1 Experiment 1: Fed indoors on lucerne hay

The quality of the diet eaten by the alpaca was slightly lower (higher fibre, but low N contents) than that of sheep, but similar *in vivo* OM digestibility (OMD) (Table 1). There were no differences ($P>0.05$) between the animal species in their apparent digestibilities of DM or GE. However, alpaca were more efficient ($P<0.05$) than sheep in digesting both NDF (47.8 v. 46.1%) and ADF (52.6 v. 50.3%).

Sheep ate significantly ($P<0.01$) more feed, both per animal and day (d) and per kg metabolic liveweight (kg LW^{0.75}), than alpaca (Table 2). The absolute

amount (g d^{-1}) of CH_4 produced by alpaca was slightly lower, but not significantly ($p>0.05$), than that by sheep (Table 2). There was no difference ($P > 0.05$) between the animal species in their CH_4 emissions expressed per unit of intakes of GE (% GEI) or digestible NDF (g kg^{-1} DNDFI) (Table 2).

No holotrich protozoa were found in the forestomach contents of alpaca (Table 2), whereas holotrichs accounted for 1.0% of the total protozoa in the rumen of sheep. Sheep had significantly higher ($P < 0.01$) counts (10^5 ml^{-1}) of both entodiniomorphids and total protozoa than alpaca.

Table 1. Chemical composition (g kg^{-1} DM) and apparent *in vitro* organic matter digestibility (%) of the forage on offer and eaten

	Forage on offer	Forage eaten	
		Alpaca	Sheep
Experiment 1: Fed indoors on lucerne hay			
Organic matter (OM)	909	912	909
Nitrogen (N)	36.5	36.7	38.3
Neutral detergent fibre (NDF)	384	394	380
Acid detergent fibre (ADF)	316	332	313
OM digestibility (OMD, %)	65.1 ¹	65.1 ²	65.0 ²
Experiment 2: Grazing on RG/WC			
Organic matter (OM)	909	905	898
Total nitrogen (N)	24	26	38
Neutral detergent fibre (NDF)	491	486	360
Acid detergent fibre (ADF)	300	303	242
Condensed tannins (CT):			
Extractable	0.5	0.6	0.5
Protein-bound	0.4	0.2	0.3
Fibre-bound	0.0	0.1	0.0
Total CT	0.9	0.9	0.8
OM digestibility (OMD, %)	72.0 ¹	67.7	76.6
Experiment 3: Grazing on Lotus			
Organic matter (OM)	926	921	919
Total nitrogen (N)	28	32	43
Neutral detergent fibre (NDF)	422	380	249
Acid detergent fibre (ADF)	344	282	199
Condensed tannins (CT):			
Extractable	13.0	12.0	25.6
Protein-bound	10.6	9.8	17.0
Fibre-bound	1.8	1.7	0.9
Total CT	25.4	23.5	43.6
OM digestibility (OMD, %)	63.0 ¹	68.6	80.0

¹ *In vitro* digestibility by NIR. ² *In vivo* values.

Table 2. Experiment 1: Fed indoors on lucerne hay. Voluntary feed intake (VFI), CH₄ emission and protozoa numbers

	ALPACA	SHEEP	S. E.	<i>P</i> value
VFI:				
DMI (g d ⁻¹)	844	1251	86	<0.01
DMI (g kg ⁻¹ LW ^{0.75})	38.8	74.0	4.0	<0.001
GEI (MJ kg ⁻¹ LW ^{0.75})	0.74	1.36	0.07	<0.001
CH ₄ emission:				
g d ⁻¹	14.9	18.8	1.7	0.13
%GEI	5.1	4.7	0.3	0.40
g kg ⁻¹ DNDFI	92.0	92.5	6.5	0.96
Protozoa (10 ⁵ ml ⁻¹):				
Holotrichs	0	0.04	0.02	0.19
Entodimorphs	2.08	3.80	0.4	<0.01

3.2 Experiment 2: Grazing on RG/WC pasture

The quality of the RG/WC diet selected by alpaca was much lower than that selected by sheep (Table 1). Accordingly, the *in vitro* OMD (%) of the sheep diet was higher than that of alpaca. As expected the condensed tannins (CT) concentrations in the forage on offer and in the diets selected were low.

Feed intake by sheep, both per animal and day (d) and per kg LW^{0.75}, were significantly ($P < 0.001$) higher than of alpaca (Table 3). The CH₄ emission of alpaca, per day (g d⁻¹), was lower ($P = 0.02$) than that of sheep (Table 3). There was a difference ($P < 0.05$) between the animal species in their CH₄ emissions expressed as a proportion of the GEI, but not ($P > 0.05$) when expressed per kg of DNDFI (Table 3).

The lack of holotrich protozoa in forestomach contents of alpaca and the small number of them in sheep were confirmed. Nevertheless, no differences between the animal species were found in their counts of protozoa (Table 3).

Table 3. Experiment 2: Grazing on RG/WC pasture. Voluntary feed intake (VFI), CH₄ emission and protozoa numbers

	ALPACA	SHEEP	S. E.	<i>P</i> value
VFI:				
DMI (g d ⁻¹)	761	1241	65	<0.001
DMI (g kg ⁻¹ LW ^{0.75})	33.5	69.8	3.3	<0.001
GEI (MJ kg ⁻¹ LW ^{0.75})	0.61	1.32	0.06	<0.001
CH ₄ emission:				
g d ⁻¹	22.6	31.1	2.2	0.02
%GEI	9.4	7.5	0.5	0.04
g kg ⁻¹ DNDFI	95.2	103.1	10.1	0.61
Protozoa (10 ⁵ ml ⁻¹):				
Holotrichs	0	0.04	0.02	0.19
Entodimorphs	4.20	4.05	0.8	0.90

3.3 Experiment 3: Grazing on birdsfoot trefoil pasture (Lotus)

As in the case of RG/WC pasture, the quality of the Lotus diet eaten by alpaca was much lower than that eaten by sheep (Table 1). The *in vitro* OMD (%) of the alpaca diet was much lower than that of sheep (Table 1). The concentration of CT in the diet selected by sheep was about twice that in the diet of alpaca or in the forage on offer (Table 1).

The VFI of sheep, both per animal and day (d) and per kg LW^{0.75}, were much higher ($P < 0.001$) than of alpaca (Table 4). The CH₄ emission of alpaca, per day (g d⁻¹), was similar ($P = 0.30$) to that of sheep (Table 4). However, per unit of intake, the CH₄ emissions of alpaca were much higher ($P < 0.001$) than of sheep (e.g. 6.4 v. 2.7 %GEI) (Table 4).

Table 4. Experiment 3: Grazing on Lotus pasture. Voluntary feed intake (VFI), CH₄ emission and protozoa numbers

	ALPACA	SHEEP	S. E.	<i>P</i> value
VFI:				
DMI (g d ⁻¹)	902	2303	119	<0.001
DMI (g kg ⁻¹ LW ^{0.75})	40.3	127.9	5.5	<0.001
GEI (MJ kg ⁻¹ LW ^{0.75})	0.77	2.53	0.11	<0.001
CH ₄ emission:				
g d ⁻¹	19.1	22.0	2.0	0.30
%GEI	6.4	2.7	0.2	<0.001
g kg ⁻¹ DNDFI	152.0	70.0	6.0	<0.001
Protozoa (10 ⁵ ml ⁻¹):				
Holotrichs	0	0.12	0.04	0.06
Entodionomorphs	4.7	16.4	1.9	<0.001

As observed in the other two forages, no holotrich protozoa were found in the forestomach contents of alpaca (Table 4) and holotrichs accounted for less than 1.0 % of the total protozoa counts in sheep. Animal species significantly differed in their counts of holotrichs ($P = 0.06$) and entodionomorphs ($P = 0.001$) (Table 4). The total concentration of protozoa (10⁵ ml⁻¹) in sheep was 3.5 times higher than in alpaca.

4.0 DISCUSSION

The higher OMD (%) for sheep diets, especially under grazing conditions, might be attributed to the selection of diets higher in N but lower in fibre than that selected by alpaca. The differences between these species in feeding preferences was very evident in this study and confirms the results of other studies (e.g. Sharp et al., 1995; Warmington et al., 1989).

The fact that on all the three forages, VFI, both per animal and day (d) and per kg LW^{0.75}, was lower in alpaca than in sheep is consistent with a lower particulate FOR in alpaca (San Martin, 1987) and the nature of the diets. DMI by sheep grazing Lotus was extraordinarily high (128 g kg⁻¹ LW^{0.75}). The Lotus pasture was prepared by weeding to a condition of almost pure stand and because herbage allowance was on the basis of leaf DM, rather than

whole herbage DM, sheep grazed almost exclusively on leaves. Thus, it is unlikely that DMI by sheep on this pasture was overestimated by miscalculations.

On all the three forages the absolute amounts (g d^{-1}) of CH_4 emitted by sheep were slightly higher (significant only on RG/WC) than those by alpaca. This can be attributed to the higher feed intakes (g d^{-1}) observed in sheep. On the other hand, except on lucerne hay, the proportions of the GEI loss in CH_4 (%GEI) were significantly lower in sheep than in alpaca. The latter is in agreement with the earlier findings by Blaxter and Clapperton (1965) of decreasing CH_4 emissions (%GEI) with increasing feed intakes and digestibilities.

Within animal species, GEI ($\text{MJ kg}^{-1} \text{LW}^{0.75}$) of lucerne hay and RG/WC were relatively similar. Despite that, the CH_4 emissions (%GEI) of both animal species on RG/WC were higher than those observed on lucerne hay. In both animal species, the intake of digestible NDF (DNDFI, g d^{-1}) on RG/WC were higher than those on lucerne hay. Since DNDFI is rich in the most methanogenic carbohydrates (Moe and Tyrrell, 1980), the higher CH_4 emissions (%GEI) observed on RG/WC may be attributed to the increased DNDFI observed both in sheep and alpaca. In fact, CH_4 emission per unit DNDFI did not differ between animal species either on lucerne hay or RG/WC. Thus, when data for lucerne hay and RG/WC were pooled within animal species, the only intake variable correlated to CH_4 emission (g d^{-1}) was DNDFI (g d^{-1}). The coefficients of correlation between these variables were 0.87 ($P=0.005$) and 0.63 ($P=0.03$) for alpaca and sheep, respectively.

The CH_4 emission (%GEI) by sheep on Lotus (2.7%) was significantly lower than that by alpaca (6.4%) and much lower than those by sheep on lucerne hay (4.7%) or RG/WC (7.5%). The depressed CH_4 emission (%GEI) by sheep on Lotus cannot entirely be attributed only to the effects of their high intakes of high quality diets (Blaxter and Clapperton, 1965), but probably also the action of some compound(s) in Lotus contributed to the low values.

The protozoal population in sheep grazing Lotus was 3.5–4.0 times higher than those in alpaca or those in sheep on the other forages. This probably was a result of the better quality of the Lotus diet, compared to that on the other forages (Jouany, 1989). It is well documented (e.g. Jouany and Lassalas, 2000) that, by virtue of the inter-species H_2 transfer, more CH_4 is lost when protozoa are present in the rumen. The depressed CH_4 emission but increased protozoa numbers observed in sheep fed on Lotus contrasts with the latter argument.

In conclusion, observations such as the differences between animal species in chemical composition of diets selected, VFI and digestibility of plant cell walls are consistent with alpaca having a lower particulate FOR than the sheep. Thus, differences between these species in particulate FOR might have been the underlying physiological mechanism responsible for the differences in CH_4 emission (%GEI).

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