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Short communication

Gas production and nitrogen digestion by rumen microbes from deer and sheep

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Abstract The rates at which gas was produced by rumen microbes from red deer (*Cervus elaphus*) and sheep were measured in vitro. The objective was to test the hypothesis that the lower frequency of rumen eructation contractions in deer, compared to sheep and other ruminants, may have been associated with a lower rate of gas production. Two sheep and two deer were held indoors, given the same feed (chaffed lucerne hay) under an identical regimen, and rumen samples obtained on 5 consecutive days from each animal. Duplicate incubations were carried out on each day with a rumen liquor inoculum from each animal in a buffered medium and a ground lucerne hay substrate. The rates of gas production were monitored for 9 h and net ammonia production determined. There were no differences between species in rate of gas production but incubation with deer liquor resulted in a 27% greater ($P < 0.01$) net conversion of substrate nitrogen (N) to ammonia compared to sheep. Gas production averaged 0.23 ml/min per g substrate between 1 and 3 h of incubation and declined to 0.15 ml/min per g substrate after 7 h. Ammonia release over the incubation period resulted in 22 and 17% of the substrate N appearing as ammonia-N from deer and sheep incubations respectively ($P < 0.01$). The difference between deer and sheep in the frequency of rumen eructation contraction sequences does not appear to be related to rates of gas production in the rumen.

Keywords deer; sheep; rumen; gas production

INTRODUCTION

This study of gas production was undertaken in response to major differences between deer and other ruminants in the frequencies of reticulo-rumen (rumen) contractions. The rumen undergoes a cyclical series of contractions which mixes digesta, aids eructation of gas, and facilitates passage of fluid material to the intestines. Contractions comprise two clearly defined types—the A or mixing sequence of contractions which commences in the reticulum and moves caudally, and the B or eructation sequence of contractions which commence in the caudal rumen and move cranially. Rumen motility has been documented in sheep, cattle, goats, and water buffalo, and in these species A sequence contractions occur at about 60 sec intervals, with one B sequence contraction for every 1 or 2 A sequences (Waghorn & Reid 1983; McSweeny et al. 1989; McSweeny & Kennedy 1992). Two studies have documented rumen motility in deer, and both have reported a much lower frequency of B sequence contractions compared to the other species. In both red deer (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*), A sequences occurred at about 40–60 sec intervals, and B sequence contractions occurred only once for every 3–5 A sequences (Dziuk et al. 1963; Stafford et al. 1992).

Although it was considered unlikely that these species would differ greatly in rumen microflora or in the rates at which gas would be produced from digestion of rumen contents, there do not appear to be any data indicating rates of gas production in red deer. For this reason an in vitro incubation trial was undertaken to compare rates of gas production from rumen inoculum taken from sheep and red deer fed identical rations under identical feeding regimens.

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The deer used in the trial were also used to determine rumen motility at an earlier time (Stafford et al. 1992).

MATERIALS AND METHODS

Two 6-year-old castrated male red deer and four 2-year-old wether sheep were held indoors in metabolism crates and fed chaffed lucerne hay over 3 weeks in August 1992. Digesta samples were taken for in vitro incubations during the third week of feeding. Particular care was taken to ensure that the four animals used as digesta donors for the incubations consumed the same feed. This was achieved by offering the chaff ad libitum and selecting two sheep which left similar proportions of stalk (refusals) to those left by the deer. Feed intakes were recorded over the 5-day incubation period, and samples of feed, refusals, and material used as substrate for incubations were retained for dry matter (DM), N, and fibre analyses.

Gas production was measured in vitro by incubating 1.25 g finely ground (1 mm sieve aperture) chaffed lucerne hay DM with 20 ml rumen liquor and 60 ml of artificial saliva (McDougall 1948). The substrate (ground hay) was from the same batch as fed to the animals, but excess stalk was removed before grinding, so that its composition was similar to that eaten by the deer and sheep. The rumen liquor was obtained 90–120 min after the animals had been given their morning feed. Although feed was always available, both deer and sheep consumed very little between midnight and 0800 h, so they were fed at 0800 h and digesta collected from an actively fermenting rumen at 0945 h. The digesta was collected into warmed, gassed (CO_2) insulated vacuum flasks and transported immediately to the laboratory. The interval between collection of digesta and its addition to the fermentation apparatus did not exceed 15 min. The digesta were squeezed through a single layer of cheese cloth, and 20 ml added to the warmed, gassed incubation vessels.

The incubations were carried out in duplicate for each animal in 250 ml flasks at 39°C in a shaking water bath (60–90/min). Each flask was connected to a manometric measuring device which enabled gas volumes to be measured at atmospheric pressure. Incubations were continued for 9.5 h, with rates of gas production recorded at 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, and 570 min.

Rumen liquor pH was measured before incubations, and liquor samples (10 ml) for ammonia determination were taken and acidified with 0.5 ml of 30% H_2SO_4 to prevent ammonia loss. At the end of each incubation period the pH was determined in the incubated digesta, and samples retained for ammonia determinations.

Analytical analyses

Fibre was analysed by sequential extraction (Robertson & Van Soest 1980), N by total combustion (Carlo Erba NA1500 Nitrogen Analyser), and NH_3 by autoanalysis (Technicon 1973).

Statistical comparisons were based on analyses of variance. Gas production was initially tested for variance between replicates. The analysis compared species, individuals, and days, and the results are presented as means ± SEM.

RESULTS

This trial was carried out in August so that the deer were eating a maintenance level of intake, (Table 1; Fennessy & Milligan 1987) and the wethers about 1.4 of maintenance (Table 1, ARC 1980). There we

Table 1 Body weights, feed dry matter (DM) offered and eaten, and rumen pH of deer and sheep. Data are means with SEM.

	Deer		Sheep		Differences	
	Mean	SEM	Mean	SEM	Species	Individuals
Body weight (kg)	120	–	49.0	–	–	–
Feed DM offered (g/day)	4165	–	1250	–	–	–
Feed DM eaten (g/day)	2443	50.3	967	18.9	***	NS
Rumen liquor pH	6.82	0.037	6.69	0.038	NS	***
Incubation media pH at 570 min	6.85	0.023	6.84	0.011	NS	NS

***$P < 0.001$; NS, not significant

no significant differences between individual deer or wethers in DM intakes. Feed offered was about 40% in excess of intake for deer and 23% in excess of intake for sheep (Table 1). The N and fibre composition of the feed actually eaten (determined from composition and amounts of feed offered and refused) was similar for the two species (Table 2). The higher concentration of N and lower concentration of neutral detergent fibre (NDF), lignin, and cellulose in material eaten, compared to the food on offer, was associated with some rejection of stalk by both deer and sheep.

The lucerne used as substrate for in vitro incubations contained similar concentrations of cellulose and lignin to that eaten by the animals, although the N content was slightly higher than that of the feed (Table 2).

Table 2 Composition of lucerne chaff offered, feed actually eaten[a] by sheep and deer, and the ground material used in in vitro incubations. Data are g/kg DM.

	Feed	Material eaten		In vitro incubations
		Deer	Sheep	
Nitrogen	28.8	32.5	31.2	29.3
NDF	518.0	463.1	483.3	419.0
Cellulose	262.4	220.7	235.1	224.0
Hemicellulose	176.7	175.1	178.5	130.6
Lignin	77.0	66.2	69.1	64.4
Ash	91.4	92.7	91.1	93.5

[a]Calculated from feed offered less feed refused
NDF, neutral detergent fibre extract

Table 3 Rate of gas production (ml/g substrate DM per min) from incubations using rumen liquor from deer and sheep. Data are means of 20 observations for each species within time periods and SEM.

Time[1]	Deer		Sheep		Difference
	Mean	SEM	Mean	SEM	
30–60	0.342	0.0131	0.294	0.0299	NS
60–120	0.273	0.0065	0.270	0.0085	NS
120–180	0.210	0.0101	0.179	0.0100	*
180–240	0.189	0.0101	0.200	0.0057	NS
240–300	0.186	0.0071	0.183	0.0095	NS
300–360	0.193	0.0065	0.192	0.0070	NS
360–420	0.173	0.0071	0.176	0.0086	NS
420–480	0.155	0.0074	0.152	0.0055	NS
480–570	0.154	0.0088	0.158	0.0068	NS

[1]Minutes from start of incubation
*$P < 0.05$; NS, not significant

The pH of rumen liquor (Table 1) was below 7.0 for all animals at the time of collection, suggesting that an active fermentation was taking place. The pH in sheep and deer digesta was similar (Table 1), but there were differences between individuals (sheep: 6.55 (SE = 0.038) and 6.82 (SE = 0.037), $P < 0.01$; deer: 6.60 (SE = 0.083) and 6.95 (SE = 0.113), $P < 0.05$). There were no differences between individuals or species in the pH of the incubated material after 570 min (Table 1).

Gas production

Rates of gas production were similar for deer and sheep (Table 3). The small difference between species between 120 and 180 min of incubation was unimportant when considered in the context of cumulative gas production over the entire incubation period. Gas production (per g substrate DM) over 540 min (from 30 to 570 min; Table 3) was 94.7 ml from sheep and 97.6 ml from deer. There were no differences between animals within species, or between replicates, in rates of gas production at any time period.

The reliability of in vitro incubations with regard to the measurements of gas production was assessed on the basis of replications within animals. Based on means for the 5 days of incubation times (Table 3), the difference between replicates (calculated for the 9 sampling periods in Table 3) averaged 3.5% (SE = 0.78) and 3.5% (SE = 1.55) for individual sheep and 3.9% (SE = 0.71) and 2.0% (SE = 0.45) for individual deer. This variation between replicates was sufficiently low as to give confidence that in vitro incubations could detect quite small differences in rates of gas production between treatments.

Ammonia production

Table 4 gives the concentration of ammonia in rumen liquor used for incubations.

Concentrations were similar in the liquor of deer and sheep. At the conclusion of incubations, the ammonia concentrations in the buffered solution were higher in deer than in sheep ($P < 0.01$). Quantitation of ammonia-N pool sizes before and after the incubation periods enabled an estimate of substrate N degradation to be calculated (Table 4). With deer inoculum, 22% of substrate N appeared as ammonia-N compared with 17% of substrate N with sheep ($P < 0.01$), although there were differences ($P < 0.05$) between the two sheep in the extent of ammonia-N production.

Table 4 Ammonia concentrations and production from in vitro incubations of rumen digesta from deer and sheep. Data are means with SEM.

	Deer		Sheep		Differences	
	Mean	SEM	Mean	SEM	Species	Individuals
Ammonia concentrations (mg ammonia-N/l)						
Rumen liquor	394	9.5	427	25.5	NS	NS
Incubation media at 570 min[a]	200	3.74	183	4.57	**	NS
Ammonia production during the incubation period[b]						
(mg ammonia-N/g substrate DM)	6.49	0.31	5.11	0.29	**	*
(mg ammonia-N/g substrate N)	221	10.6	174	9.9	**	*

[a]Rumen liquor diluted 1:3 with buffer
[b]Nett production
*$P < 0.05$; **$P < 0.01$; NS, not significant

DISCUSSION

The principal finding in this study was that rates of gas production from in vitro incubations using a rumen microbial inoculum from deer and sheep yielded similar rates of gas production, so that differences between the two species in the frequency of B sequence contractions are likely to result from factors other than the need for eructation. The second observation was that incubations based on deer inoculum yielded a greater net release of ammonia-N than that from sheep, suggesting that the deer may be better able to degrade feed protein than sheep, despite a similar fermentation rate (based on gas production). The microbes from deer rumen liquor may have a different substrate affinity to those from sheep.

It is difficult to interpret differences in B sequence contraction frequency between species because there is insufficient information on function and regulation of rumen motility and because motility is affected by diet as well as time spent eating, ruminating, and resting. However, several trials have been reported where chaffed lucerne hay has been fed to sheep, cattle, and deer and rates of rumen A and B sequence contractions measured over 24 h. Numbers of A and B contractions/min were 1.0 and 0.58 in sheep, 1.2 and 0.66 in cattle, and 1.15 and 0.28 in red deer (Waghorn & Reid 1983; Stafford et al. 1992). Comparable values for goats fed chopped wheaten hay were 1.0 and 0.63 and for water buffalo fed roughages A and B contractions were 1.2 and 0.6/min (McSweeny et al. 1989; McSweeney & Kennedy 1992). Hence sheep, goats, cattle, and water buffalo all have a similar and more frequent occurrence of B sequence contractions than red deer. In view of the similar rates of in vitro gas production measured here for red deer and sheep, it would appear that different processes may be responsible for initiation of B sequence contractions in deer and sheep. In white-tailed deer, eructation occurred with most B sequence contractions (Dzuik et al. 1963) so that the need for eructation may be the principal determinant of frequency in red deer, but not in sheep, cattle, goats, and water buffalo.

The frequency with which B sequences occurred in deer may be regulated by different processes to those in sheep and other ruminants. For example, insufflation experiments with cattle have shown that the capacity to eructate far exceeds that required under normal feeding conditions (Dougherty et al. 1965). When cattle are fed dry forages the rate of gas production is about 20–30 l/h (Colvin et al. 1958; Hoernicke et al. 1964), the rumen digestion pool is about 6 kg DM, and the space or gas "pocket" above the rumen digesta raft is about 20 l (Waghorn 1991). Hence the hourly rate of gas production is similar to the volume of the gas cap which would suggest that the need for eructation is infrequent, and that a major function of B sequence contractions may include the mixing of digesta. The suggestion that B sequence frequency in deer could be governed primarily by the need for eructation could be examined by rumen insufflation and monitoring of motility.

From a nutritional view point, the higher proportion of substrate N appearing as ammonia-N in incubations from deer (0.22) compared to sheep (0.17) may be important. These proportions are less than the total de-amination because some substrate N will have been incorporated into microbial crude protein. The total DM degraded during in vitro incubations, estimated on the basis of gas production (100 moles gas from 58 moles hexose fermentation; Hungate 1966), was about 0.37 of the ground lucerne

chaff substrate for both deer and sheep, so that the higher net ammonia-N production in deer suggests a greater proteolytic activity of deer microflora. However, this is not supported by in vivo comparisons of N kinetics in deer and sheep. A comparison between red deer and sheep fed roughage diets suggested a similar DM and N digestion for the two species (Maloiy & Kay 1971), and more recently Domingue et al. (1991) reported similar rumen ammonia concentrations and irreversible loss rates from sheep and red deer fed chaffed lucerne hay.

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