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Digestion and rumen metabolism of red clover and perennial ryegrass/white clover forages by red deer

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SUMMARY

Red clover (RC) and perennial ryegrass (PRG)-based forages were cut fresh during late spring/early summer and fed at similar levels of dry matter (DM) intake to rumen fistulated castrated red deer kept indoors in metabolism pens. RC contained higher concentrations of total N and non-protein cell contents than PRG and lower concentrations of fibre. Rumen pool size and fractional outflow rates of liquid (13·3 *v.* 15·1 %/h) and of particulate matter (2·5 *v.* 3·9 %/h) were lower for deer fed RC than PRG. Apparent digestibility of energy and fibre, rumen fibre fractional degradation rate and rumen fractional disappearance rate of non-protein cell contents were all higher for RC than for PRG deer. Nitrogen retention was similar for deer fed both forages. However, the concentration, pool size and outflow of ammonia from the rumen, together with urinary N excretion, were all much greater for deer fed RC than those fed PRG. The acetate:propionate ratio in rumen volatile fatty acids (VFA) was lower for the RC than the PRG group. It was concluded that the greater fibre digestion in deer fed RC was due to a faster rumen fractional degradation rate and a longer particulate mean retention time in the rumen, and that the very rapid outflow of water from the rumen relative to particulate matter in deer fed RC (5·5:1 *v.* 3·8:1) may explain why deer are not susceptible to rumen frothy bloat when grazing RC. One reason for the greater voluntary feed intake (VFI) of deer grazing RC than those grazing PRG may be due to its greater concentration of protein and non-protein cell contents and their more rapid degradation and removal from the rumen.

INTRODUCTION

Production of venison is a new and rapidly growing form of animal production in New Zealand (Ataja *et al.* 1992). In New Zealand, venison production is based on red deer grazing conventional mixed perennial ryegrass (*Lolium perenne*; 75%) and white clover (*Trifolium repens*; 25%) pastures. The growth of conventional pastures is often reduced in summer due to moisture stress (Korte *et al.* 1987). This can result in a feed deficit, as voluntary feed intake (VFI) is seasonal in red deer and peaks in summer (Barry *et al.* 1991). This feed deficit is a potential problem especially during lactation, as red deer are late calvers (late spring/early summer) and are therefore grazing pastures of low nutrient quantity/quality during lactation.

Tetraploid red clover (RC: *Trifolium pratense*) is known as a summer crop with a deep taproot to resist summer drought, and is highly preferred by red deer (Hunt & Hay 1990). Red deer fawns reared on hinds grazing pure RC swards during lactation had significantly higher growth rates and weaning weights compared with control animals grazing conventional perennial ryegrass (PRG) pasture (Niezen *et al.* 1993). Liveweight gains of weaner deer grazing RC were significantly greater than those grazing PRG pasture, with stags on RC being 7 kg heavier at 1 year of age and hinds 3 kg heavier than animals grazing PRG (Semiadi *et al.* 1993). The greater VFI of RC has been shown to contribute to this superior performance (Niezen *et al.* 1993, Semiadi *et al.* 1993). The aim of the present study was to compare the digestion, rumen metabolism, rumen outflow rate and nitrogen retention of red deer fed RC and PRG forages at the same level of DM intake.

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Table 1. *Composition (g/kg DM) of fresh-cut forages fed to rumen fistulated red deer stags (mean and, standard error, S.E.)*

	Red clover ($n = 4$)	Ryegrass/white clover ($n = 4$)
Dry matter (g/kg fresh)	124 ± 7·2	179 ± 5·4
Organic matter	886 ± 4·3	892 ± 5·1
Total fibre*	303 ± 10·5	479 ± 30·7
Hemicellulose	94 ± 1·7	203 ± 14·8
Cellulose	181 ± 9·1	258 ± 17·6
Lignin	28 ± 0·71	18 ± 0·45
Total nitrogen	43·9 ± 5·49	29·4 ± 3·86
Non-protein cell contents†	309 ± 7·1	229 ± 5·5
Gross energy (kJ/g DM)	18·7 ± 0·28	18·3 ± 0·20

* Neutral detergent residue.
† Calculated as 100 − ash % − NDF % − (N % × 6·25).

MATERIALS AND METHODS

Experimental design

This study was based on a factorial crossover design of eight animals randomly allocated to one of two forages (RC and PRG) and with two feeding periods, with the diets crossed over for the second period. Each animal grazed its assigned pasture for 14 days, prior to being fed in metabolism crates during a 7-day adjustment period, followed by a 7-day collection of urine and faeces. Cr-EDTA was continuously infused into the rumen during the last 5 days of the collection period, after which the rumen was emptied and sampled.

Animals and housing

Eight hand-reared castrate male red deer were used, aged 3–5 years and weighing 117·7 kg (W) ± 3·2 kg (S.E.). All deer were fistulated in the rumen and fitted with a permanent rubber cannula (83 mm i.d.). The animals were kept in metabolic crates similar to those described by Milne *et al.* (1978). The crates were housed in a well ventilated building with artificial light set at 14 h light and 10 h dark (prevailing natural day length), provided with water *ad libitum* and free access to a multi-mineral salt block placed in each feed bin.

Diets

Fresh, rapidly growing tetraploid RC (cv. Pawera) and PRG pastures were cut daily during late spring/early summer 1990. The RC and PRG forages contained respectively 24 and 29% of white clover and mean lengths were 310 and 325 mm. The dry matter (DM) fed (g/kg $W^{0.75}$/day) was equivalent between the forages and was based on the VFI of the PRG pasture measured during the initial 7-day adjustment period. The DM content of each feed offered was rapidly determined by drying in a microwave oven. Fresh forage was delivered from overhead belt feeders at 30 min intervals, for complete 24-h periods, during all the time the deer were indoors.

Marker infusion

The inert liquid marker Cr-EDTA was prepared by the method of Binnerts *et al.* (1968) and adjusted to a pH of 6·5–7·0. The Cr-EDTA was made up to 20 litres with a final Cr concentration of 2 mg/g of solution. Following a priming dose of 40 g into the rumen, the marker solution was continuously infused into the rumen for 5 days at a rate of 23–25 g/h, with the exact infusion rate being determined for each animal. The infusion was administered by a peristaltic pump (PLG-multipurpose pump, Desaga, Heidelberg, Germany).

Sample collection

Feed, feed refusals and faeces were weighed daily during the collection period, and subsamples were taken and stored at −20 °C. Another daily subsample was taken of each material for duplicate DM determination in a forced-draught oven (100 °C). Urine was weighed daily from buckets containing sufficient 25% sulphuric acid to maintain pH < 3·5. After the collection period, all frozen subsamples were bulked within animals, thoroughly mixed and re-sampled, then freeze-dried and ground through a 1 mm mesh sieve and used for laboratory analysis.

The rumens were emptied while the deer were lightly sedated with xylazine (Rompun, Bayer AG, Germany). Rumen contents were weighed, thoroughly mixed and subsampled before returning the warmed digesta back to the rumen. The whole process was completed in 15–20 min per animal and the animals remained standing throughout the procedure.

Rumen fluid samples for volatile fatty acids (VFA), NH_3-N and Cr were taken twice daily (10.00 and 15.00 h) for 3 days from a tube that passed through the bung of the rubber cannula, attached to a perforated brass cylinder covered with a nylon mesh (80 μ aperture; Swiss Screens, Australia). The length of the sampling tube was adjusted so that the sampling apparatus hung near the middle of the rumen contents. The first 20 ml of rumen fluid was discarded at each sampling. Rumen fluid was also obtained when the rumen was emptied by squeezing mixed digesta through nylon mesh (80 μ aperture). Fluid for NH_3-N and VFA was acidified, deproteinized and centrifuged as described by Domingue *et al.* (1991*a*).

Analytical procedures

Samples of feed, feed refused, faeces and rumen digesta were analysed for cell wall constituents by the

Table 2. *Intake and digestion of dry matter, energy, fibre and non-protein cell contents in rumen fistulated red deer stags fed fresh forages (mean and standard error, S.E.)*

	Red clover ($n = 8$)	Ryegrass/white clover ($n = 8$)	S.E.
Dry matter			
Intake (g/kg $W^{0.75}$/day)	52·9	49·1	0·76
Apparent digestibility	0·798	0·735	0·0078
Organic matter			
Intake (g/kg $W^{0.75}$/day)	46·9	42·3	0·75
Apparent digestibility	0·820	0·745	0·0072
Energy			
ME intake (kJ/kg $W^{0.75}$/day)	651	530	9·8
Apparent energy digestibility (%)	0·795	0·726	0·0075
Fibre			
Intake (g/kg $W^{0.75}$/day)	15·8	22·3	0·86
Rumen pool size (g/kg $W^{0.75}$)	9·54	12·15	0·662
Apparent digestibility	0·749	0·673	0·0141
Fractional disappearance rate (%/h)	7·27	7·68	0·447
Fractional degradation rate (%/h)	4·75	3·77	0·349
Non-protein cell contents			
Intake (g/kg $W^{0.75}$)	16·2	11·2	0·21
Rumen pool size (g/kg $W^{0.75}$)	4·00	4·00	0·152
Fractional disappearance rate (%/h)	17·75	8·45	0·803

detergent method (not including the amylase) of Robertson & Van Soest (1980), total nitrogen (N) by the Kjeldahl method, gross energy by adiabatic bomb calorimetry (Gallenkamp Autobomb, UK), and organic matter by ashing overnight at 555 °C. Total N of urine was also determined by the Kjeldahl method. Cr concentration was determined by atomic absorption spectrometry. NH_3-N and VFA were determined as described by Domingue *et al.* (1991*a*).

Calculations

Fractional outflow rate (FOR) and fractional disappearance rates (FDPR) from the rumen were calculated as shown below:

$$\text{FOR}(\%/\text{h}) = \frac{\text{Marker infusion rate (mg/h)} \times 100}{\text{Rumen pool size (mg)}} \quad (1)$$

$$\text{FDPR}(\%/\text{h}) = \frac{\text{Intake (g/h)} \times 100}{\text{Rumen pool size (g)}} \quad (2)$$

Cr-EDTA was used to calculate rumen liquid FOR and lignin to calculate particulate FOR; in the latter case, faeces lignin excretion was used to substitute for infusion rate in Eqn (1) on the assumption that there was minimal post-ruminal degradation. Rumen fractional degradation rate (FDR) was calculated from Eqn (3), which shows disappearance from the rumen to be the sum of both degradation and outflow. In calculating rumen FDR of total fibre, it was assumed that total fibre left the rumen on the same particle as lignin and therefore had the same FOR.

$$\text{FDPR} = \text{FDR} + \text{FOR} \quad (3)$$

Rumen NH_3-N outflow was calculated by multiplying rumen NH_3-N pool size by the FOR of Cr-EDTA. Rumen mean retention time (MRT) was calculated as the reciprocal of FOR (Faichney 1975). Metabolizable energy (ME) intake was calculated as digestible energy intake × 0·82.

Statistical analyses

Mean values and the standard errors of the means (S.E.M.) are presented. The values for NH_3-N and VFA are the means of seven observations/animal, since there were no significant differences ($P > 0{\cdot}10$) between the day, time of day or type of sampling (rumen emptying or by sampling apparatus). Univariate analysis of variance of repeated measures (Gill 1986; Genstat 1988) was used to remove between-animal variation before analysing for dietary and period differences. All dietary comparisons in Tables 2–5 involved 5 D.F. for error. Means were compared by the method of least significant difference (Snedecor & Cochran 1980).

RESULTS

RC contained lower concentrations of total fibre, cellulose and hemicellulose than PRG, but higher concentrations of total N, non-protein cell contents

Table 3. *Rumen pool size, rumen fractional outflow rate and rumen mean retention time for liquid and particulate matter in rumen fistulate red deer stags fed fresh-cut forages (mean and standard error, S.E.)*

	Red clover ($n = 8$)	Ryegrass/ white clover ($n = 8$)	S.E.
Rumen pool size (g/kg $W^{0.75}$/day)			
Total*	228	251	8·2
Dry matter (DM)	24·3	25·7	1·28
Liquid	203	226	7·0
g DM/g DM intake/day	0·46	0·52	0·019
DM of rumen digesta (%)	10·7	10·1	0·28
Fractional outflow rate (%/h)			
Cr-EDTA	13·3	15·1	0·433
Lignin	2·52	3·92	0·287
Cr-EDTA/lignin	5·51	3·84	0·347
Mean retention time (h)			
Cr-EDTA	7·74	6·70	0·273
Lignin	43·1	26·2	4·60

* DM + liquid.

Table 4. *Nitrogen (N) balance and apparent N digestibility in rumen fistulated red deer stags fed fresh forages (mean and standard error, S.E.)*

	Red clover ($n = 8$)	Ryegrass/white clover ($n = 8$)	S.E.
N fluxes (g N/kg $W^{0.75}$/day)			
Intake	2·36	1·38	0·021
Faecal excretion	0·39	0·31	0·013
Urinary excretion	1·64	0·79	0·042
N balance			
g N/kg $W^{0.75}$/day	0·32	0·27	0·038
g N/100 g DMI*	0·61	0·57	0·084
Apparent N digestibility (proportion N eaten)	0·831	0·765	0·0078
Urine N (proportion N eaten)	0·702	0·596	0·0206

* Dry matter intake.

and lignin (Table 1). Apparent digestibility of dry matter, organic matter and energy ($P < 0·01$) and of fibre ($P < 0·05$) were greater for RC than PRG, as was metabolizable energy intake ($P < 0·001$) (Table 2). There was no difference between the two forages in rumen fractional disappearance rate of total fibre, but fibre fractional degradation rate tended to be greater for deer fed RC ($P < 0·10$; Table 2). The fractional disappearance rate of non-protein cell contents from the rumen was markedly greater in deer fed RC than in deer fed PRG ($P < 0·01$).

Total rumen pool size and rumen liquid pool size were lower for deer fed RC than PRG ($P < 0·10$; Table 3). Rumen liquid fractional outflow rates were high for both diets. Fractional outflow rates of both Cr-EDTA and lignin from the rumen were lower for deer fed RC compared to PRG ($P < 0·05$), whilst the ratio Cr-EDTA/lignin fractional outflow rates was much higher for deer fed RC ($P < 0·05$). Rumen mean retention time was higher for deer fed RC than those fed PRG ($P < 0·05$).

The intake, faecal excretion and urinary excretion of N were all greater for deer fed RC than PRG ($P < 0·01$; Table 4). However, N retention did not differ ($P > 0·10$) between deer fed the two forages. Whilst apparent N digestibility was highest for RC ($P < 0·01$), urinary N as a proportion of N intake was also much higher on this diet than for deer fed PRG ($P < 0·01$).

Total VFA concentration ($P < 0·01$) and the molar proportions of propionate ($P < 0·01$), iso-butyrate ($P < 0·01$), *n*-valerate ($P < 0·01$) and iso-valerate ($P < 0·01$) were all higher in the rumen fluid of deer fed RC than PRG, whilst molar proportions of acetate ($P < 0·05$), *n*-butyrate ($P < 0·10$) and the acetate:propionate ratio ($P < 0·01$) were lower for deer fed RC (Table 5). The concentration ($P < 0·01$), pool size ($P < 0·01$) and outflow ($P = 0·06$) of ammonia from the rumen were all greater for deer fed RC than PRG.

DISCUSSION

Under the late spring/early summer growing conditions during which this study was done, RC was of higher apparent DM and energy digestibility than PRG, probably due to its higher concentrations of protein and non-protein cell contents and lower concentration of fibre. The higher concentration of non-protein cell contents in RC probably reflects higher concentrations of soluble carbohydrate and pectin, both of which are higher in white clover than PRG and both of which are rapidly digested in the rumen (Ulyatt & MacRae 1974). Rumen fractional outflow rate of liquid was very high for both diets, as observed by Domingue *et al.* (1991*b*), who found rumen liquid fractional outflow rate to be higher for deer (16%/h) than for sheep and goats fed a lucerne chaff diet (10%/h). Despite its higher concentrations of protein and non-protein cell contents, RC had a slower rumen fractional outflow rate (and longer mean retention time) of both liquid and particulate matter than did PRG, and thus showed rumen outflow characteristics of concentrate-type diets, which have lower rumen fractional outflow rates than forage diets (Barry *et al.* 1985).

Table 5. *Rumen ammonia concentration and outflow, and the concentration and molar proportions of volatile fatty acids (VFA) in the rumen fluid of rumen fistulated red deer stags fed fresh forages (mean and standard error, S.E.)*

	Red clover ($n = 8$)	Ryegrass/white clover ($n = 8$)	S.E.
VFA			
Total VFA (mmol/l)	119·3	96·1	2·14
Molar concentration (mol%)			
Acetate	67·7	68·8	0·25
Propionate	19·5	18·5	0·09
n-butyrate	8·2	9·0	0·23
iso-butyric	1·70	1·44	0·039
n-valeric	1·05	0·86	0·030
iso-valeric	1·91	1·51	0·047
Acetate:propionate ratio	3·48	3·73	0·022
Ammonia			
Concentration (mg NH_3-N/l)	321	215	8·4
Rumen pool (g NH_3-N)	2·19	1·66	0·065
Rumen outflow (mg NH_3-N/day)	290	246	11·7

The very rapid rumen outflow of liquid relative to particulate matter in deer fed RC may have relevance to the aetiology of rumen frothy bloat, which is normally severe in cattle grazing RC. Bloat is caused by an accumulation of soluble protein in the rumen (Mangan 1959) and soluble protein content is particularly high in RC (Barry & Forss 1983). The very rapid outflow of liquid relative to particulate matter in deer fed RC may also act as a rapid removal mechanism for soluble proteins, thus preventing their accumulation in the rumen and explaining why we have never observed a single case of bloat over a 3-year period in red deer grazing RC.

Surprisingly, there was no difference in fibre fractional disappearance rate (degradation plus outflow) between deer fed RC and PRG. The greater apparent fibre digestibility in deer fed RC therefore appears to be due to a faster rumen fractional degradation rate and longer mean retention time, allowing more time for fibre digestion.

As N retention was similar for deer fed each forage, despite the greater N intake from RC, it seems that both diets were supplying enough amino acids to sustain maximum N retention in these 3–5 year old castrate male deer. The higher rumen ammonia concentration, pool size and outflow rate in deer consuming RC, together with the greater apparent N digestibility and much greater urinary N loss, all suggest that the N in this diet was very readily soluble in the rumen, rapidly fermented to ammonia and large quantities excreted in the urine. Iso-butyric and iso-valeric acids are also the end products of rumen de-amination of valine, leucine and iso-leucine (Van Soest 1982); higher concentrations of these VFA in the rumen fluid of deer fed RC also confirms a higher rate of de-amination on this diet. Thus the N in RC was not efficiently used in the present study, and may not promote optimum N use in high producing deer, such as lactating hinds and rapidly growing young deer.

The greater VFI of lactating (Niezen *et al.* 1993) and of growing deer (Semiadi *et al.* 1993) grazing RC than of similar deer grazing PRG under field conditions cannot be explained by rate of rumen outflow (which was slower for RC) or by fibre FDPR (which did not differ between diets). Rather, the higher contents of protein and non-protein cell contents in RC and their more rapid rumen disappearance on this diet may be one reason for the greater VFI, thus explaining the lower rumen pool size (DM + liquid) in deer fed RC and allowing more opportunity for increased feed intake.

High acetate:propionate ratios in rumen VFA may indicate inefficient utilization of acetate above maintenance (and hence ME) under some circumstances (Black *et al.* 1987). Hence, the lower acetate:propionate ratio on RC may lead to improved utilization of ME by growing deer.

In conclusion, the digestion characteristics of RC are such as to give greater energy and fibre digestibility, hence greater energy absorption than PRG and lower total rumen pool size, thus allowing an opportunity for increased voluntary DM intake. However, N retention was inefficient in RC, and legumes with reduced N solubility in the rumen need to be evaluated for deer production. Plants containing low concentrations of condensed tannins such as sulla (*Hedysarum coronarium*) and chicory (*Cichorium intybus*), may be important in this regard (Barry 1989; Terrill *et al.* 1992).

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