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Digestion, rumen fermentation and chewing behaviour of red deer fed fresh chicory and perennial ryegrass

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SUMMARY

Pure chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*) forages were cut and fed fresh at Palmerston North, New Zealand, during March 1993 to castrated male red deer kept indoors in metabolism crates. Chicory contained lower levels of dry matter, higher levels of ash, and had a higher ratio of readily fermentable:structural carbohydrate than perennial ryegrass. Apparent digestibility of organic matter was highest for chicory (0·81 *v.* 0·72), but cellulose apparent digestibility was highest for perennial ryegrass (0·71 *v.* 0·59). Relative to perennial ryegrass, the rumen fluid of deer fed chicory contained higher concentrations of protozoa, ammonia and total volatile fatty acids (VFA) but had a lower pH at 15.00 h. Chicory-fed deer had higher rumen VFA molar proportions of *n*-butyrate and a higher acetate:propionate ratio. Total eating time and chews during feeding/g dry matter intake were similar for deer fed the two forages, but deer fed chicory spent much less time ruminating (33 *v.* 270 min/day) and had fewer rumination boluses (38 *v.* 305/day). It was concluded that the low rumination time may indicate rapid disintegration of chicory in the rumen to < 1 mm critical particle size, and that particle breakdown and rumen fractional outflow rate should be measured in future experiments with deer fed on chicory.

INTRODUCTION

Chicory (*Cichorium intybus*) is a herbaceous perennial member of the Asteraceae family native to Europe and parts of Asia, Africa and the Americas. It was used in New Zealand (NZ) as an animal forage as early as 1915, when it was included in pasture mixes because of its high concentration of minerals (Cockayne 1915). Later trials in NZ showed that chicory established well and exhibited good drought resistance characteristics, with excellent summer dry matter (DM) production under rotational grazing (Lancashire 1978) and on dryland sites, although there was considerable variation in performance between individual plants (Rumball 1986). AgResearch Grasslands, Palmerston North, began selecting chicory lines for superior forage characteristics, and 'Puna' chicory was approved for commercial release, national listing and certification in 1985 (Rumball 1986). Puna chicory has been shown to grow at rates up to 200 kg DM/ha per day during spring (Hare & Rolston 1987). Several trials have shown Puna chicory to have a superior feeding value for sheep, cattle and deer, with the feeding value of chicory for lambs being similar to that of white clover and lucerne (Brown 1990).

In order to grow deer to reach target liveweights of 92 kg (50 kg carcass) by 12 months of age (Ataja *et al.* 1992), nutritional strategies including the feeding of specialist crops must be used. Chicory produces large quantities of DM during spring, summer and autumn, which coincides well with deer feed requirements and with the peak in the seasonal VFI cycle of temperate deer (Milne *et al.* 1978; Domingue *et al.* 1991*a*). As long as the sward remains predominantly vegetative, chicory is strongly preferred by deer (Hunt & Hay 1990) and can produce weaner stag autumn liveweight gains of > 300 g/day (K. Kusmartono, personal communication), as well as superior liveweight gains in lactating hinds and fawns (Niezen *et al.* 1993). The objective of the present experiment was to establish reasons for the superior feeding value of chicory relative to perennial ryegrass (*Lolium perenne*).

MATERIALS AND METHODS

Experimental design

An indoor trial was conducted with red deer (*Cervus elaphus*) fed either fresh perennial ryegrass (*Lolium*

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perenne L. cv. Nui), or chicory (*Cichorium intybus* L. cv. Grasslands Puna). Factors investigated included apparent digestibility, the composition of rumen fluid, and eating, ruminating and resting activity. The trial was carried out at Massey University for 37 days from 22 February 1993 to 30 March 1993, and was divided into an adjustment phase (10 days), a digestibility trial (10 days) and a jaw recording period (17 days).

Animals and housing

Ten castrated, hand-reared stags each fitted with an 83 mm diameter rumen cannula, with mean initial and final liveweights (±S.D.) of 140 (±17·4) and 134 (±14·0) kg respectively were used. The animals were accustomed to handling and being indoors in specially designed deer metabolism crates (Milne *et al.* 1978) fitted with automatic feeders to allow half-hourly feeding. One side of the cage was moveable and could be used to adjust the floor area. Prior to being brought inside, the deer were grazing perennial ryegrass/white clover pastures adjacent to the animal house.

Animals were randomly assigned to treatment groups based upon liveweight. A 10-day initial adjustment period allowed the animals to adjust to indoor conditions, to the two diets offered and to handling procedures, including restriction of movement caused by reducing the floor area.

Diets

The chicory was sown in December 1992 and was a pure, vegetative crop. The perennial ryegrass, sown in 1991, was from a pure sward 10 cm in height. Fresh forage was cut daily at 14.00 h. During the adjustment and digestibility periods, the overhead feeders were set to deliver every 30 min to achieve the 'steady state' conditions required, with filling of the overhead feeders occurring at 08.00 and 16.00 h. During jaw recording the animals were fed twice daily at 08.00 and 16.00 h using the troughs. The animals were fed as close as possible to a constant dry matter intake (DMI) of 2·1 kg/day in all three periods of the experiment. This was done by taking triplicate samples of feed offered at 08.00 h and following cutting of forage at 14.00 h for dry matter (DM) determination (100 °C; 18 h).

Digestibility, rumen fluid and jaw recording

Feed offered and refused was weighed and faeces quantitatively collected and weighed daily over the period 4–13 March. Duplicate 200 g samples of feed offered were taken daily and pooled at −20 °C. Each animal's residual feed was collected and pooled at −20 °C (per animal). Duplicate subsamples were taken of pooled feed and feed residues for freeze-drying, grinding and chemical analysis. Faeces were collected daily and separated from hair and residual forage, pooled per animal and stored at −20 °C. Later they were homogenized, triplicate samples were taken for DM determination (100 °C; 48 h) and duplicate samples taken for freeze-drying, grinding and chemical analysis.

Two days before rumen fluid sampling was due to commence, rumen probes covered with synthetic (80 μm pore size) polyester fibre (Swiss Screens, Sydney, Australia) were inserted into the cannula of each animal. On 8, 10 and 11 March, samples of rumen fluid were taken at 10.00 and 15.00 h from nine animals only, as one animal (chicory treatment) strongly resisted attempts to sample rumen fluid. A total of 50 ml of fluid was taken, 5 ml for protozoa counting, 20 ml for ammonia analysis, 5 ml for VFA analysis and 15 ml for pH measurement. The sample for protozoa counting was sterilized in formal saline and stored at 4 °C. The sample for pH was analysed within one hour of sampling, and the samples for VFA and ammonia analysis were added to protein precipitant (Domingue *et al.* 1991*b*), centrifuged (1600 *g*) and stored at −20 °C.

A 2-day adjustment period allowed the animals to adjust to twice-daily feeding, while remaining on constant DM intake. Five complete 24-h periods of jaw activity were recorded for each of eight animals (four on perennial ryegrass: four on chicory), starting at 08.00 h each day, from 15 to 29 March. Both animals and recordings were inspected frequently during each 24-h period. A chart recording speed of 25 mm/min was used for 4 consecutive days to measure eating and ruminating time, followed by one day at 100 mm/min to measure frequency of chewing during both eating and rumination and to measure rumination boluses.

To prevent the deer turning around during recording sessions and tangling the recording lines, the width of the cage was reduced by means of the movable wall. The degree of restriction was such that while the animals could not turn around, they could stand, lie down, eat or drink without hindrance.

The recording system was similar to that described by Stafford *et al.* (1992, 1993) for counting jaw activity. A 4-channel chart recorder (Graphtec Linearecorder WR3701–4Hx1, Japan) allowed simultaneous recording of jaw activity from each of four animals (two on perennial ryegrass; two on chicory). Jaw movements were sensed as pressure changes in a partially inflated rubber bag held under the jaw with a halter. The bag was a section of bicycle inner tube, closed off at one end, the other end sealed and cemented over a flexible nylon pipe (3·5 mm i.d.) joined to a 0·8 m section of coiled rubber infusion tubing (CenVet, Australia) which accommodated

animal movement. Nylon tubing connected the rubber tubing to an electronic pressure transducer (Statham, ADCG, Hong Kong) mounted outside the cage. The transducer was connected via a pre-amplifier to the pen recorder. Upon completion of these measurements, the recording equipment was transferred to a further two deer consuming each forage.

The records were analysed for periods of eating, ruminating or idling (resting) using the following conventions. Eating bouts were considered to be a minimum of 5 min duration and continuous unless interrupted for more than 10 min by a bout of rumination or idling. Rumination bouts were defined as involving a minimum of three boluses regurgitated and chewed, with bouts separated by a minimum of 5 min idling or feeding. 'Pseudo-rumination' was defined as attempts at rumination, consisting of rumination bouts of less than three boluses, and/or erratic rumination with inconsistent and infrequent chewing. Idling bouts were periods of no jaw movements.

Laboratory analyses

Following freeze-drying, all feed, residue and faeces samples were ground to pass a 1 mm sieve (Willey Mill, USA). Organic matter content was measured by ashing in a furnace of 500 °C for 16 h and total nitrogen (N) was determined by the Kjeldahl technique. Neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, cellulose and lignin contents were determined following the procedures of Goering & Van Soest (1970). Hot water soluble carbohydrate (HWSC) and pectin were extracted using boiling water and ammonium oxalate respectively, as described by Bailey & Ulyatt (1970). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Watson Victor Ltd, UK). VFA were determined by gas chromatography (HRGC 5300, Carlo Erba Instruments, Italy) and ammonia-N was distilled from sodium tetraborate as described by Domingue *et al.* (1991 *b*). The pH of rumen fluid was determined on a PHM 61, Laboratory pH meter (Radiometer, Copenhagen, Ltd). Extractable and bound condensed tannins were determined by the modified butanol/HCl procedure of Terrill *et al.* (1992), and extractable condensed tannins were also determined by the vanillin/HCl procedure of Broadhurst & Jones (1978). Protozoa were counted using a haemocytometer (Hawksley Mod-Fuchs Rosenthal, BS 748, UK) with standard counting chamber (0·2 mm depth).

Statistical analysis

Tests for significance of differences between treatment means were carried out by one-way analysis of variance. In the case of rumen fluid, mean values for each animal were calculated over the three sampling days, for each sampling time, and these were then statistically analysed.

RESULTS

The dry matter content of chicory was much lower than that of perennial ryegrass ($P < 0·001$). Chicory contained significantly higher amounts of ash ($P < 0·001$), hot water soluble carbohydrate ($P = 0·051$) and pectin ($P < 0·01$) in its DM than perennial ryegrass and contained significantly lower amounts of NDF, ADF, hemicellulose, cellulose and gross energy ($P < 0·01$; Table 1). This gave a significantly higher ratio of readily fermentable carbohydrate:structural carbohydrate (RFC:SC; $P < 0·05$) for chicory compared with perennial ryegrass. Chicory had a non-significantly lower nitrogen content than perennial ryegrass and a slightly higher lignin content. Chicory and perennial ryegrass both contained trace amounts of condensed tannins (CT), but these were consistently higher for chicory.

Digestibility values (Table 2) were high for both forages, with digestibility of dry matter (DM; $P < 0·05$), organic matter (OM: $P < 0·05$) and gross energy ($P < 0·01$) being higher for chicory than for perennial ryegrass. NDF and ADF digestibility were both lower for chicory than for perennial ryegrass ($P < 0·01$).

Table 1. *The chemical composition of perennial ryegrass and chicory (g/kg)*

	Perennial ryegrass ($n = 2$)	Chicory ($n = 2$)	S.E. (2 D.F.)
Dry matter ($n = 13$)	209	110	0·9
Ash	117	188	1·4
Nitrogen	32·3	28·9	1·61
Hot water soluble carbohydrate (*a*)	80	134	8·9
Pectin (*a*)	12	98	0·5
NDF	467	238	8·2
ADF	251	178	2·0
Hemicellulose (*b*)	216	60	0·4
Cellulose (*b*)	229	148	0·4
Ratio RFC:SC (*a*/*b*)*	0·21	1·15	0·097
Lignin	22	30	2·7
Gross energy (MJ/kg DM)	18·5	16·5	0·11
Condensed tannin			
Extractable†	1·05	1·66	0·145
Extractable‡	0·65	0·97	0·083
Protein-bound‡	1·17	1·83	0·178
Total‡	1·82	2·80	0·195

* Readily fermentable carbohydrate:structural carbohydrate.
† Vanillin–HCl method.
‡ Butanol–HCl method.

Table 2. *Apparent digestibility of dry matter, organic matter, gross energy and fibre in fresh perennial ryegrass and chicory fed to red deer*

	Perennial ryegrass ($n = 5$)	Chicory ($n = 5$)	S.E. (8 D.F.)
Dry matter	0·685	0·752	0·0145
Organic matter	0·723	0·806	0·0123
Gross energy	0·678	0·766	0·0149
Fibre			
NDF	0·674	0·569	0·0149
ADF	0·640	0·518	0·0191
Hemicellulose	0·715	0·684	0·0154
Cellulose	0·707	0·591	0·0213
Lignin	−0·328	0·181	0·0413
Metabolizable energy (MJ/kg DM)	10·3	10·4	0·04

This was principally due to a lower digestibility of cellulose in chicory ($P < 0·01$), with hemicellulose digestibility being similar for the two forages. The digestibility of lignin in chicory was significantly greater than that for perennial ryegrass ($P < 0·001$), with the latter being negative due to the formation of artefact lignin.

Rumen fluid pH at 15.00 h was markedly lower for deer consuming chicory than those on perennial ryegrass ($P < 0·001$), but there was no significant difference between forages at the 10.00 h sampling time ($P > 0·05$). Rumen ammonia concentration followed the opposite trend, being significantly greater for chicory at the 10.00 h sampling ($P < 0·05$), but not significantly different from perennial ryegrass at 15.00 h ($P > 0·05$). Protozoa numbers were significantly greater in the rumen fluid of chicory-fed animals than perennial ryegrass-fed animals at both sampling times ($P < 0·05$; Table 3).

Total VFA concentration was significantly higher for the chicory-fed animals at both 10.00 h ($P < 0·01$), and 15.00 h ($P < 0·001$) (Table 4). There was no significant difference between treatments in acetic acid molar proportions at either sampling time ($P > 0·05$), but propionic acid proportions were significantly lower for the deer fed chicory ($P < 0·01$). Consequently, the rumen acetate:propionate ratio was higher for the chicory-fed animals ($P < 0·01$). *N*-butyric acid proportions were significantly higher for the chicory treatment ($P < 0·01$). Proportions of iso-butyric, iso-valeric and *n*-valeric acids were lower in the rumen fluid of chicory-fed deer than the perennial ryegrass-fed deer at 15.00 h ($P < 0·05$) but not at 10.00 h.

Total eating time, number of eating bouts and chews per unit DMI were all slightly but non-significantly lower for deer consuming chicory ($P > 0·05$) than perennial ryegrass (Table 5). Chews per minute eating were higher ($P < 0·01$) and chews per unit fresh matter intake ($P = 0·057$) were significantly lower for the deer consuming chicory. Total rumin-

Table 3. *The pH, ammonia concentration and protozoa counts in the rumen fluid of red deer fed fresh perennial ryegrass or chicory. (Mean values for three sampling times per animal)*

	Perennial ryegrass ($n = 5$)	Chicory ($n = 4$)	S.E. (7 D.F.)
pH			
10.00 h	6·80	6·74	0·142
15.00 h	6·73	6·30	0·048
Ammonia concentration (mg N/l)			
10.00 h	91·3	166·3	16·49
15.00 h	172·9	182·8	11·07
Protozoa (/ml × 10^5)			
10.00 h	7·32	13·13	1·579
15.00 h	7·94	16·30	2·048

Table 4. *The concentration and molar proportions of volatile fatty acids (VFA) in the rumen fluid of red deer fed fresh perennial ryegrass or chicory. (Mean values for three sampling times per animal)*

	Perennial ryegrass ($n = 5$)	Chicory ($n = 5$)	S.E. (8 D.F.)
Total VFA concentration (mm/l)			
10.00 h	60·0	74·5	0·33
15.00 h	61·7	85·1	0·29
VFA molar proportion (%)			
Acetic			
10.00 h	69·0	68·2	0·33
15.00 h	68·1	68·2	0·37
Propionic			
10.00 h	18·0	15·5	0·34
15.00 h	18·2	16·4	0·24
n-butyric			
10.00 h	9·2	12·1	0·30
15.00 h	9·1	11·2	0·28
Iso-butyric			
10.00 h	1·4	1·4	0·06
15.00 h	1·6	1·2	0·06
Iso-valeric			
10.00 h	1·5	1·8	0·07
15.00 h	1·7	1·4	0·07
n-valeric			
10.00 h	0·9	1·0	0·04
15.00 h	1·3	1·1	0·05
Acetic:propionic ratio			
10.00 h	3·84	4·42	0·105
15.00 h	3·75	4·21	0·063

Table 5. *Eating, ruminating and idling (resting) behaviour in red deer fed fresh perennial ryegrass or chicory*

	Perennial ryegrass ($n = 4$)	Chicory ($n = 4$)	S.E. (6 D.F.)
Eating behaviour			
Eating time (min/day)	379	361	43·6
Eating bouts (/day)	19·3	15·3	2·97
Chews/min	81·9	100·4	3·89
Chews/g fresh	3·94	1·98	0·587
Chews/g DMI	18·7	17·9	3·49
Ruminating behaviour			
Ruminating time (min/day)	270	33	12·5
Rumination bouts (/day)	14·4	3·9	0·84
Rumination boluses (/day)	304·6	37·8	13·65
Chews/bolus ruminated	51·3	27·4	1·07
Chews/min	58·1	31·3	2·87
Pseudo-rumination bouts (/day)	1·50	3·85	0·0872
Idling time (min/day)	791	1045	43·3

ating time, number of boluses ruminated, number of rumination bouts and chews per bolus ruminated were all markedly lower for the deer fed chicory ($P < 0·001$). Chewing rate during rumination was lower for animals fed chicory ($P < 0·001$), whilst bouts of pseudo-rumination ($P < 0·01$) were significantly higher for the chicory animals than those consuming the perennial ryegrass diet.

DISCUSSION

The ratio of readily fermentable carbohydrate: structural carbohydrate in chicory (1·15:1·00) is similar to that found for white clover (1·17:1·00; Ulyatt & MacRae 1974; Ulyatt *et al.* 1976), but the ash content of chicory (188 g/kg CM) is substantially higher than that found for most perennial forages (90–110 g/kg DM). The greater concentration of CT in chicory relative to perennial ryegrass could be important when considering protein digestion. Low concentrations of CT (0·17% DM) can reduce protein solubility in the rumen and hence can reduce the incidence of rumen bloat in cattle (Waghorn & Jones 1987).

The high digestibility values for DM, OM and gross energy in both forages can be explained by the highly vegetative state of both swards. The higher DM, OM and gross energy digestibilities for chicory can be explained by its high ratio of readily fermentable carbohydrate:structural carbohydrate and probable more rapid degradation in the rumen than perennial ryegrass. The low digestibility of cellulose in chicory may be related to rumen pH; cellulose digestibility is pH-dependent and optimized at pH 6·7 (Van Soest 1982). Higher quality feeds usually produce a slightly lower rumen pH, as was shown for chicory, and this may be a reason why cellulose digestibility was decreased. More research is needed with chicory-fed deer to obtain pH values at intervals over 24 h periods, to establish whether rumen pH declines to very low levels.

The population densities of rumen ciliate protozoa are strongly influenced by the animal's diet, being increased by high concentrations of both soluble carbohydrate and insoluble protein (Jouany 1989; Michalowski 1989). Higher protozoa populations in deer fed chicory may therefore be explained by its high concentration of readily fermentable carbohydrate, and its slightly higher concentration of CT than in perennial ryegrass.

Diets with a high ratio of readily fermentable: structural carbohydrate normally produce rumen fermentations with a lower acetate:propionate ratio, as found by Freudenberger *et al.* (1994) when they compared red clover with perennial ryegrass/white clover pasture fed to deer. However, the reverse was found for chicory in the present study, with this feed having a higher ratio of readily fermentable:structural carbohydrate than perennial ryegrass, yet producing a rumen fermentation with a higher acetate: propionate ratio and greater proportions of *n*-butyrate. The relative molar proportions of VFA in rumen liquor are influenced by the rumen liquid dilution rate (Harrison *et al.* 1975), rumen protozoa (Van Soest 1982) and pH (Esdale & Satter 1972). A higher liquid dilution rate (i.e. fractional outflow rate), caused by intraruminal infusion of artificial saliva, was found to increase the acetate:propionate ratio in sheep (Harrison *et al.* 1975). The higher ash content of chicory may lead to a higher liquid dilution rate in a similar way to infusion of artificial saliva, by increasing the rumen osmotic pressure. Therefore a higher rumen dilution rate in deer fed chicory could

explain the occurrence of a higher acetate:propionate ratio, despite a higher dietary readily fermentable carbohydrate:structural carbohydrate ratio, and rumen dilution rate should be measured in future studies of chicory digestion.

Acetate and *n*-butyrate are the principal fermentation products of protozoa (Van Soest 1982) and the higher protozoan populations in the chicory-fed deer might also be a contributing factor towards the higher acetate:propionic acid ratio, and higher *n*-butyrate concentration found for the chicory-fed deer. High absorption of acetate from the rumen relative to propionate lowers the efficiency of utilization of metabolizable energy for growth (Black *et al.* 1987). Therefore in terms of the efficiency of utilization of absorbed energy, chicory may offer little advantage over perennial ryegrass.

A key finding in this study was the low rumination times, rumination bouts and number of boluses ruminated for deer fed chicory, relative to deer fed perennial ryegrass. The number and rate of chews during rumination by deer fed chicory was also much lower than for deer fed perennial ryegrass. Feed particulate matter in the rumen must be reduced to below a critical particle size (< 1 mm for deer; Domingue *et al.* 1991*a*) in order for it to have a high probability of being cleared from the reticulo-rumen via the reticulo-omasal orifice (Reid *et al.* 1977). Feed particulate matter is reduced in particle size by two main processes: chewing during eating and chewing during rumination (Ulyatt *et al.* 1984). Therefore the low rumination times in deer fed chicory suggest rapid disintegration of particulate matter in the rumen to < 1 mm in size. Crush & Evans (1990) suggested that silicon is present in chicory at very low levels compared with ryegrass, and silicon can alter the mechanical properties of herbage. The low silicon concentration and high readily fermentable carbohydrate:structural carbohydrate ratio may be reasons why chicory is more readily broken down than perennial ryegrass.

If chicory rapidly disintegrates in the rumen, then particulate fractional outflow rate from the rumen should also be high. Further research is needed to establish rumen fractional outflow rate of liquid and particulate matter and to determine the rate of particle size reduction in the rumen in deer fed chicory. Clearance of digesta from the reticulo-rumen has long been recognized as a major process determining both intake and nutritive value of forages (Black *et al.* 1982), and rapid disintegration and outflow of digesta from the rumen would help to explain the high VFI and high levels of production found in deer grazing chicory (Niezen *et al.* 1993; K. Kusmartono, personal communication). There is thus a need to make these detailed nutritional measurements with deer fed chicory in future experiments.

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