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Rumen fermentation characteristics are influenced by feeding frequency in sheep fed forage chicory and perennial ryegrass at two feeding levels

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Abstract

The objective of this study was to investigate the effects of feeding frequency and level of intake on rumen fermentation parameters of sheep fed chicory or perennial ryegrass. Fresh, vegetative chicory or perennial ryegrass were fed twice daily at 0900 h and 1630 h in the first period, and hourly in the second period to eight rumen-fistulated wethers at 1.3 and 2.2 times their maintenance metabolisable energy requirements. Rumen fluid pH, ammonia and volatile fatty acid (VFA) concentrations at 0900, 1000, 1100, 1200, 1300 and 1500 h were determined. When fed hourly ($P < 0.05$), but not twice-daily, forage type and feeding level significantly affected rumen pH. Differences in total VFA concentrations between forages and feeding levels were influenced by feeding frequency. Ammonia concentrations were always lower in sheep fed chicory than ryegrass ($P = 0.001$). Differences in rumen pH, ammonia and total VFA concentrations attributed to either forage species, level of intake or their interactions and were more likely to be detected when sheep were fed hourly, compared to twice-daily, as a result of the reduction in diurnal variation of these parameters. Manipulation of feeding frequency and/or feed intake can modulate the responses of ruminal metabolism to fresh forage diets.

Keywords: feeding frequency; rumen fermentation; chicory; perennial ryegrass; feeding level; sheep

Introduction

Animal nutrition research often involves the evaluation of both feeds and feeding practices. Rumen fermentation characteristics are a good indicator in evaluating nutritional treatments (French & Kennelly 1990). However, feed and forage evaluation trials often employ a feeding regime with one or two meals per day, resulting in one or two peaks in rumen fermentation activity and subsequent nutrient flows for the ruminants to utilise.

Feeding frequency has a wide range of influences on ruminants, including feed intake and digestion (Robles et al. 2007), the excretion of faeces and urine (Minson & Cowper 1966), animal performance and product quality (Gibson 1981, 1984; Zali & Ganjkhani 2007; Pulido et al. 2009), animal behaviour (DeVries et al. 2005), and rumen fermentation profiles (French & Kennelly 1990; Le Liboux & Peyraud 1999). Alternative forages, especially herbs such as chicory and plantain, sown as pure cultures or with legumes are increasingly being used for dairy and meat production systems in New Zealand (Lambert et al. 2004). Controlled digestion studies to evaluate the nutritive value of these new forages have mostly employed twice-daily feeding at feeding levels close to maintenance energy requirements (Hoskin et al. 1995; Tinworth et al. 1999; Swainson & Hoskin 2006). Yet relatively little information appears available in the literature on either the effects of frequency of feeding or level of intake of different fresh forage diets on rumen digestion and fermentation profiles.

Knowledge of the effects of both contrasting feeding frequencies and levels of intake are required to improve the interpretation of nutrition research

findings when diets are compared at a specific feeding frequency and level of intake. This information may also have relevance for translating research results into practice, as grazing management can influence feeding patterns in practical farm situations (Chilibroste et al. 1997).

The objective of this study was to investigate how feeding frequency and level of feeding affects rumen fermentation characteristics, namely rumen fluid pH, ammonia and volatile fatty acid (VFA) concentrations, of sheep fed chicory and perennial ryegrass, as part of a larger trial investigating methane emissions from these forages (Sun et al. 2012).

Materials and methods

Experimental design

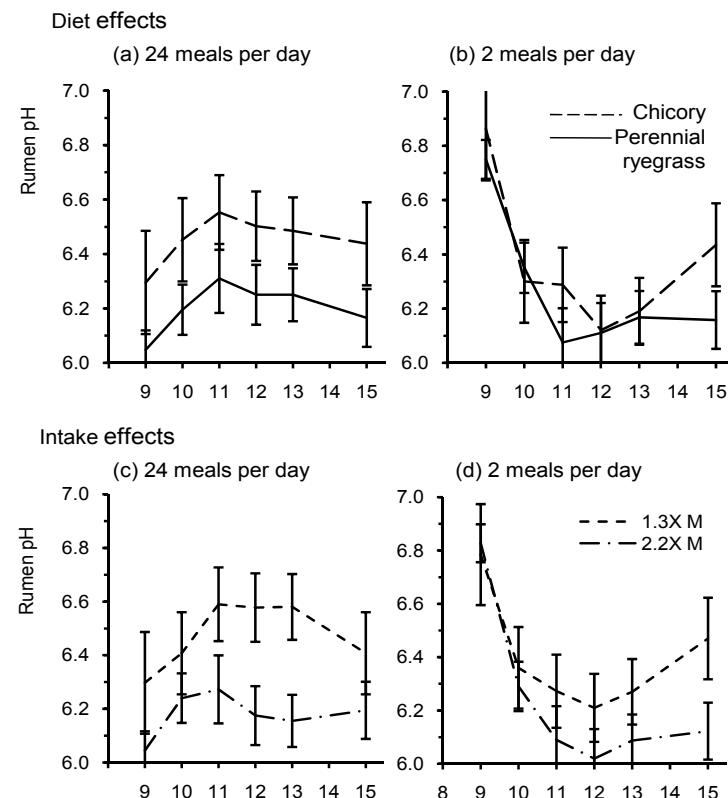
The study was conducted during autumn in April and May 2009 at AgResearch Grasslands (Palmerston North, New Zealand) with eight rumen fistulated Romney wether sheep under animal ethics approval granted by the AgResearch Grasslands Animal Ethics Committee. The sheep were randomly divided into two groups of four. One group grazed chicory and another perennial ryegrass pasture for a seven-day adaptation period. Then the sheep were placed in pens for six days for adaptation to feeding levels and to housing conditions. Two sheep in each group were fed twice daily at 1.3 times their maintenance metabolisable energy (ME) requirements (Australian Agricultural Council 1990) and another two at 2.2 times maintenance. The sheep were held in metabolic crates from Day 14 to Day 27 and meal allocation set as follows: from Day 14 to Day 21 they were fed hourly and from Day 22 to Day 27, twice daily at

Table 1 Chemical composition (g/kg dry matter) of forage chicory and perennial ryegrass diets.

Component	Chicory	Perennial ryegrass
Ash	196	127
Crude protein	114	197
Hot water-soluble carbohydrates	153	114
Pectin	75	10
Neutral detergent fibre	239	423
Acid detergent fibre	188	218
Hemicellulose	51	204
Cellulose	106	191

0900 h and 1630 h. Six rumen samples were taken on Day 19 when sheep were fed hourly and on Day 27 when fed twice daily at 0900 h and 1630 h for measurement of pH, VFA and ammonia concentrations. Thus, the study comprised of a 2-meal and a 24-meal trial, and had a 2×2 factorial design for each trial with two forage species (chicory and perennial ryegrass) and two level of intake requirement ($1.3\times$ and $2.2\times$ maintenance ME) as experimental factors.

Figure 1 Rumen fluid pH of wethers fed chicory or perennial ryegrass at 1.3 or 2.2 times maintenance metabolisable energy requirement (M) fed hourly (24 meals a day) (a) and (c) or twice daily (b) and (d) ($n = 4$ sheep per sub group). Error bars indicate the standard error of the mean.



Forages

Forage chicory (*Cichorium intybus* cv. Choice) or perennial ryegrass (*Lolium perenne* cv. Quartet) were in the vegetative stage during both grazing and indoor feeding periods of the experiment. Chicory had re-grown for six weeks prior to the experiment and was approximately 50 cm in height; ryegrass had four week of regrowth and was approximately 35 cm in height. Forages were harvested for indoor feeding using a sickle bar mower, stored at 4°C and fed fresh as described by Sun et al. (2011). The composition of forage chicory and perennial ryegrass is presented in Table 1.

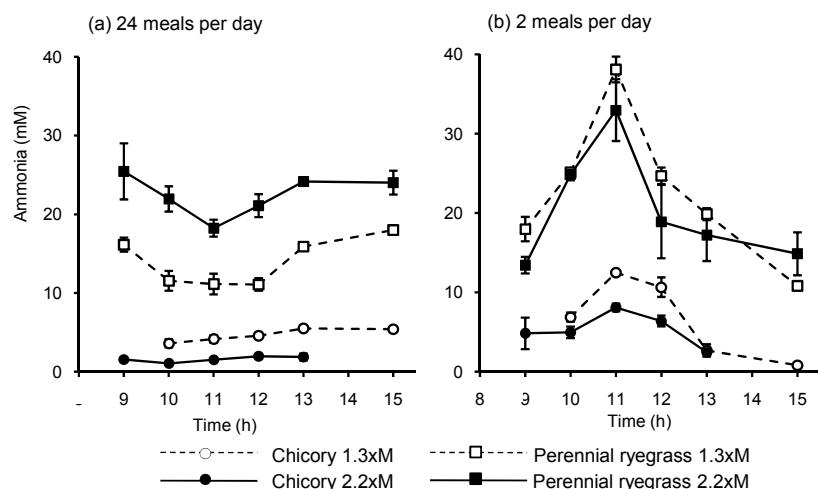
Animals and feeding

The eight wethers were about 20 months of age and weighed 48 ± 2.9 (mean \pm SD) kg. They were drenched with a broad spectrum anthelmintic and followed the same protocol as described by Sun et al. (2011), prior to the experiment. During the grazing period, forage was supplied at generous levels to allow *ad-libitum* intake, followed by the designated feeding levels when sheep were housed indoors. When the sheep were fed twice daily, two equal meals were provided at 0900 h and 1630 h; when fed hourly, 24 equal meals were provided using automated overhead belt-feeders. Drinking water was always available. Feed allowance was calculated for each feeding level based on the forage ME content estimated using near infrared reflectance spectroscopy (FeedTECH, AgResearch, Palmerston North, New Zealand) as described by Sun et al. (2010).

Rumen fermentation parameters

Rumen fluid samples (~20 ml) were taken from all animals via the rumen fistula at 0900 h (before feeding), 1000 h, 1100 h, 1200 h, 1300 h and 1500 h. Rumen fluid pH was measured using a PHM210 standard pH meter (Radiometer Analytical, France) immediately after sampling. A 1.8 mL subsample was centrifuged at 20,000 g for 5 minutes at 4°C and 0.9 ml of supernatant added to 0.1 mL of acid solution containing 200 μ L/mL ortho-phosphoric acid and 20.0 mM 2-ethyl butyric acid as described by Sun et al. (2011) and stored at -20°C until analysed. The acid-treated samples were thawed, centrifuged again as described above and the supernatant used for VFA and ammonia concentration determination. VFA concentration was determined by gas chromatography (Sun et al. 2011), ammonia concentration using the nitroprusside method of Weatherburn (1967).

Figure 2 Ammonia concentration in the rumen fluid of wethers fed chicory or perennial ryegrass at 1.3 or 2.2 times maintenance metabolisable energy requirement (M) fed hourly (24 meals a day) (a) or twice daily (b) ($n = 2$ sheep per sub group). Error bars indicate the standard error of the mean.



Statistical analysis

All variables were analysed by a repeated measurement model, with the power model as correlation within sheep across time, using REML in GenStat (Payne et al. 2010). Forage, feeding level and their interaction were considered as having fixed effects in the model. The analyses were conducted for the two meal or the 24 meal periods separately. Direct comparisons between feeding regimes were not possible due to the confounding of period and feeding regime in this trial.

Results

The consumed forage chicory and perennial ryegrass that the sheep consumed (Table 1) were of typical quality (Barry 1998). Chicory had less crude protein, more hot water-soluble carbohydrates and pectin, and less neutral detergent fibre than ryegrass. The quality of forages offered in the two periods was similar.

pH

The main effect of feeding frequency on pH was a lower variation for hourly feeding compared with twice daily meals (Fig. 1). When fed hourly, sheep fed chicory had a higher rumen fluid pH than ryegrass ($P = 0.07$) (Fig. 1a), however, there were no differences in overall rumen fluid pH between forages with twice daily feeding ($P = 0.35$) (Fig. 1b) except at 1500 h. Rumen fluid pH was lower in sheep fed high versus low intakes (6.18 versus 6.48 , $P = 0.07$) (Fig. 1c) when fed 24 meals per day, but there were no effects when fed twice daily, except at 1500 h ($P = 0.35$) (Fig. 1d). There was no interaction between forage species and feeding level on rumen

fluid pH under either hourly ($P = 0.40$) or twice-daily ($P = 0.88$) feeding.

Ammonia

Rumen fluid ammonia concentrations were always lower in sheep fed chicory than ryegrass (7.7 versus 27.7 mM, respectively; $P = 0.001$). When the wethers were fed hourly, rumen ammonia concentrations remained relatively constant and there was a strong interaction between forage species and feeding level for rumen ammonia concentrations ($P = 0.005$) (Fig. 2a). At the lower feeding level, the mean concentrations of ammonia in the rumen fluid of wethers were lower when fed chicory (5.4 mM) compared with perennial ryegrass (17.8 mM), and at the high feeding level, this difference was even larger (2.3 versus 27.4 mM).

When two meals were given daily, regardless of forage fed, the ammonia concentration reached a peak at around 1100 h, two hours after feed was given (Fig. 2b), but the peak was much lower ($P < 0.001$) for chicory (13.7 mM), compared with perennial ryegrass (41.1 mM). Feeding level ($P = 0.58$) and its interaction with forage species ($P = 0.34$) did not significantly affect the rumen fluid ammonia concentrations.

VFA

Feeding frequency affected the profile of total VFA concentrations (Fig. 3), but had little effect on molar proportions of individual VFAs. Under the hourly feeding regime, forage species ($P = 0.05$), feeding level ($P = 0.005$) and their interaction ($P = 0.005$) all significantly affected the total VFA concentrations, but there were no significant effects for any of these factors when sheep were fed twice daily. With hourly feeding, rumen fluid total VFA concentrations were similar (98.6 mM) for chicory at both feeding levels, but for ryegrass, rumen fluid total VFA concentrations were greater at the high intake level (119.5 mM) compared with the lower intake level (92.2 mM).

The molar proportions of acetate and propionate in total VFA and the ratios of acetate to propionate were similar regardless of forage species and feeding level. However, the proportion of *n*-butyrate was higher ($P < 0.01$) for chicory than for perennial ryegrass during both frequencies of feeding.

Discussion

This study is unique in its investigation of how rumen fermentation parameters were influenced by both

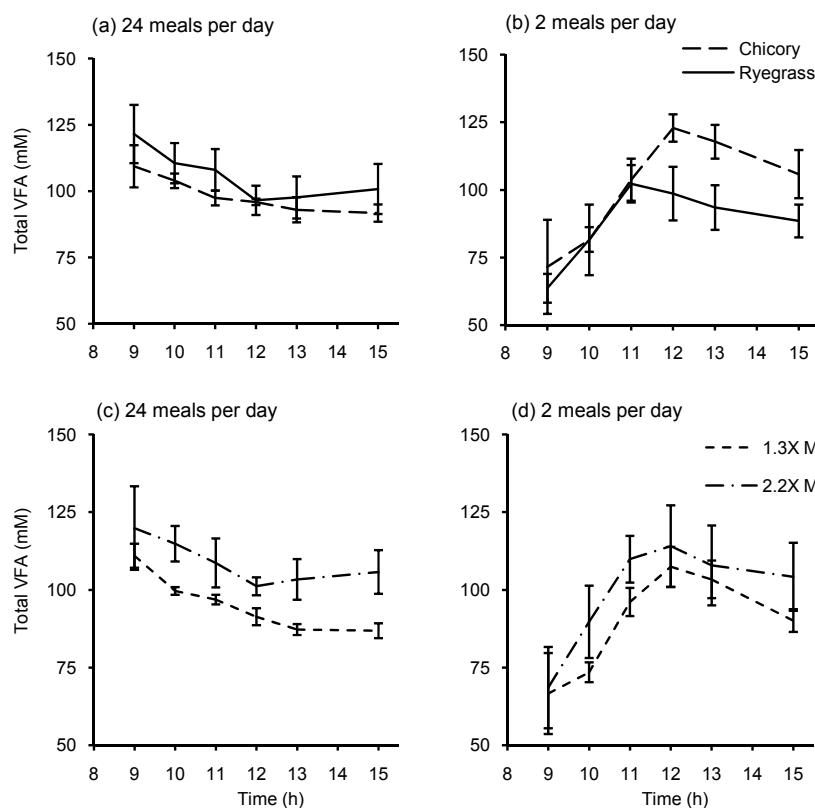
feeding frequency and level of feed intake when sheep were fed fresh forages. The availability of equipment to provide hourly meals (Minson & Cowper 1966) enabled researchers to minimise the diurnal variation in digestive processes. Since then, high-frequency feeding regimes have been often implemented in studies as an experimental design feature to steady the flux of nutrients from digestion for measurement purposes (Hoskin et al. 2001), or to explore opportunities to modify production from ruminants (Burt & Dunton 1967; French & Kennelly 1990). However, in spite of the abundance of published studies describing the effect of feeding frequency on ruminal or productive aspects (Burt & Dunton 1967; Gibson 1981), the number of studies in which fresh forages have been studied is relatively scarce.

In general, feeding fresh forages hourly *versus* twice-daily to sheep in this study reduced the diurnal variation in the rumen fermentation parameters measured. Increasing the feeding frequency from two to 12 meals per day resulted in less variation in the rumen pH of cows fed concentrates (French & Kennelly 1990). Even increasing the frequency of feeding from two to four meals per day has reduced the diurnal variation in both rumen pH and ammonia in dairy cows (Shabi et al. 1999), while increasing the frequency of concentrate feeding from two to 12 times a day decreased ammonia concentrations and increased apparent digestibility in steers (Alvarez Almora et al. 2012). Together, these results suggest important modulatory effects of feeding frequency occurring over a large range of ruminant species and dietary conditions. Increasing the feeding frequency in this study reduced the diurnal variation in rumen ammonia concentrations across different forages and levels of feeding.

In this study, sheep fed chicory had lower ammonia concentrations in rumen fluid than those fed ryegrass. This result is in contrast to the study by Hoskin et al. (1995), who fed red deer with chicory and observed that ammonia concentration in the rumen was higher, compared to that of animals fed perennial ryegrass. The difference in rumen ammonia concentrations in our study was most likely due to the crude protein content of chicory being much lower than ryegrass (11.4 *versus* 19.7% of DM) (Table 1).

Differences in rumen pH, rumen ammonia and total VFA concentrations attributed to either forage

Figure 3 Volatile fatty acid (VFA) concentration in rumen fluid of wethers fed chicory or perennial ryegrass at 1.3 or 2.2 times maintenance metabolisable energy requirement (M) fed hourly (24 meals a day) (a) and (c) or twice daily (b) and (d) ($n = 4$ sheep per sub group). Error bars indicate the standard error of the mean.



species, level of intake or their interactions were more likely to be detected under an hourly- compared with twice-daily feeding regime in the present study. This is probably due to the increased statistical power resulting from a reduction in diurnal variation of these parameters. While it is important that the feeding regime assumed in a particular research protocol is designed to meet the demands of the science, it is important to understand the effects of feeding frequency when interpreting results from studies conducted with a common diet, but at different feeding frequencies.

Beyond controlled indoor feeding situations, the understanding of feeding frequency on aspects of ruminal metabolism is important for interpreting grazing studies and may be relevant for optimising feeding management practices on commercial farms. Yet relatively few grazing studies include measurements of rumen fermentation parameters, either by use of rumen-fistulated animals or stomach-tubing intact animals to obtain rumen fluid (Tavendale et al. 2006). Pastoral livestock production involves a variety of feeding regimes, best demonstrated in New Zealand as set stocking *versus* rotational grazing. With intensive rotational grazing, such as in dairy systems where cows receive new breaks of pasture twice a day (Holmes et al. 2002), or beef systems using within-day break feeding with

portable electric fencing such as TechnoGrazing™ (Kiwitech International, Bulls, New Zealand) systems where the number, timing and size of meals can be controlled (Charlton & Wier 2001). Thus, the effect of such imposed feeding patterns on rumen fermentation need to be understood in order to interpolate research results and to identify ways to manage rumen conditions with the aim of increasing productivity and reducing environmental impact from grazing livestock (Blaxter & Clapperton 1965; Beukes et al. 2011).

In conclusion, hourly- versus twice-daily feeding reduced the diurnal variation in rumen fermentation parameters. Increased feeding frequency and/or level of feed intake increased the likelihood of detecting differences in rumen fermentation parameters between fresh forages. These results highlight the need for careful experimental design and interpretation of findings from rumen metabolism research conducted under specific feeding regimes because results obtained from a specific frequency and/or level of feeding cannot necessarily be extrapolated to other feeding frequencies or levels of intake. This study has emphasised that manipulation of feeding frequency and/or feed intake can modulate the responses of ruminal metabolism to diet. Further research is needed to characterise the mechanisms underlying the interactions between diets, feed intake and feeding frequency. Given that feeding frequency and intake can be influenced by grazing management strategies (Hodgson 1990), rumen fermentation responses for fresh forages should be characterised as close as possible to practical feeding situations to allow the optimisation of nutritional managements in pastoral systems.

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