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Ruminal fermentation characteristics from sheep offered a fresh grazing allocation of a ryegrass-based pasture either in the morning or in the afternoon

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ABSTRACT

Eight rumen-fistulated wethers were used to examine the effects of PM (16:00 h) versus AM (08:00 h) allocation of spring herbage from pasture on aspects of ruminal metabolism that lead to increased utilisation of nutrients. The experimental period consisted of two phases; adaptation to diets and intensive sampling. During the adaptation phase, total volatile fatty acids (VFA) and ammonia concentrations were greater for the PM group, whereas the ratio of non-glucogenic to glucogenic VFA did not differ. These results suggest greater fermentation from forage that had similar concentrations of crude protein, neutral detergent fibre, and water soluble carbohydrates, but greater organic matter digestibility (84.1 vs. 82.3%). During the intensive sampling phase, the mean total VFA and ammonia concentrations did not differ among groups, but the 24-hour pattern varied considerably for all variables measured. Unseasonal weather conditions during the trial were associated with smaller differences in water soluble carbohydrate accumulation than expected. Based on our results, at least four hours of sunshine were needed to obtain diurnal differences greater than 20 g per kg herbage DM. The implication of these findings on the implementation of nutritional management strategies warrants further investigation.

Keywords: grazing; ruminal metabolism; herbage allocation; ammonia; volatile fatty acids.

INTRODUCTION

Improvements in ruminal nitrogen (N) utilisation, largely in response to improved energy to N ratios and/or reduced proteolysis, have been key nutritional aspects for improving livestock efficiency of N utilisation from temperate forages. Perennial ryegrass (*Lolium perenne*)-based pastures often supply excessive N and moderate to low concentrations of water soluble carbohydrates (WSC),primarily from fructans, which restricts optimisation of N capture by the grazing ruminant (Miller *et al*., 2001). As WSC supply the most readily available source of energy for grazing ruminants, increases in the supply of WSC or a more balanced consumption of fermentable carbon (C) to N in diets, have resulted in enhanced nutrient capture in the rumen. The fermentation process leading to this enhancement is often characterised by reduced ammonia loads (Lee *et al*., 2002; Tavendale *et al*., 2006; Trevaskis *et al*., 2004) and increased proportions of glucogenic VFA precursors, such as propionate relative to acetate and butyrate (Gregorini *et al*., 2008; Lee *et al*., 2002; Tavendale *et al*., 2006). The reduced ammonia load is associated with a potential reduction of urinary urea N excretion (Kingston-Smith & Theodorou, 2000). The enhancement in glucogenic precursors is associated with the supply of additional energy available to the animal as glucose (Huntington & Archibeque, 1999).

Under most grazing conditions, selective breeding (Lee *et al*., 2002) and management strategies (Orr *et al*., 2001) have proven effective in increasing the concentration of WSC in ryegrasses. Among the grazing management strategies explored, diurnal accumulation of phosynthates accounted for consistent contrasts between WSC and crude protein (CP) concentrations in herbage (Cosgrove *et al*., 2009). As the day progresses, the rate of accumulation of WSC in the plant exceeds the rate of breakdown for growth and respiration (Fulkerson & Donaghy, 2001). Consequently, diurnal increases in dry matter (DM) and WSC concentrations in the plant, frequently at the expense of neutral detergent fibre (NDF) and CP concentrations (Delagarde *et al*., 2000) often result in greater organic matter digestibility (OMD) and overall herbage nutritive value (Gregorini *et al*., 2008). The objective of this study was to evaluate the effects of afternoon versus morning allocation of fresh herbage on measures of ruminal metabolism in sheep, namely digesta pH, ammonia and VFA. It was hypothesized that ruminal ammonia concentrations would be lesser, and glucogenic to lipogenic VFA concentrations would be greater, from sheep grazing a fresh strip of pasture during the afternoon, as a consequence of a more balanced fermentable C to fermentable N ratio offered during this time of the day.

MATERIALS AND METHODS

Eight rumen-cannulated Romney wethers with a mean live weight ± standard error, of 52.3 ± 1.8 kg were used to examine the effects of morning (AM; from 08:00 h; n = 4) versus afternoon (PM; from 16:00 h; n = 4) grazing allocation of a spring pasture on aspects of ruminal metabolism. Wethers grazed on the same strip for a 24-hour period and were offered the same daily allowance. The experiment was conducted during the spring of 2009 in late October and November. Wethers were offered a generous portion of fresh herbage daily, monitored closely by measurements of post-grazing height (6.1 ± 0.4 cm). The experimental period consisted of two phases; adaptation to diets and intensive sampling of ruminal digesta samples. During the adaptation phase, samples from all animals were collected two hours after herbage allocation on days 1, 8, and 14 via ruminal cannulae. After the 2-week adaptation phase, ruminal digesta samples from all animals were collected according to the following schedule: Samples were collected consecutively at 08:00 h (prior to new AM herbage allocation), 12:00 h, 16:00 h (prior to new PM herbage allocation), 20:00 h, 24:00 h, 04:00 h, 10:00 h, 14:00 h, 18:00 h, 22:00 h, 02:00 h, and 06:00 h. Ruminal culture pH was obtained using a MeterLab® (PHM210, Radiometer Pacific Limited, Copenhagen), calibrated immediately prior to each set of readings. Ammonia concentrations were determined using a colorimetric procedure (Weatherburn, 1967) and VFA concentrations were determined by gas liquid chromatography (Attwood *et al.*, 1998).

Weather data were obtained from the local weather station (AgResearch, Grasslands Research Centre, Palmerston North). A total of 85 mm of rainfall occurred during the experimental period, with daily air temperatures of 15.5 ± 0.45°C maximum, 6.7 ± 0.88°C minimum, and 3.9 ± 0.78 hours/day sunshine. Only five out of 16 days had more than five hours of sunshine per day. Herbage samples from the ryegrass-based pasture (81% perennial ryegrass, 19% white clover, DM basis) were collected during both phases at ~08:00 h and 16:00 h, immediately prior to new herbage allocation. Estimates of plant chemical composition including organic matter (OM), WSC, CP, NDF, acid detergent fibre (ADF) and OMD were obtained by near infrared reflectance spectroscopy (FeedTECH™, Palmerston North, New Zealand). Estimates of DM intake were obtained from pre- and post-grazing herbage mass estimates using an electronic rising plate meter (RPM) (Farmworks Precision Farming Systems, Fielding, New Zealand). Aproximately 50 RPM readings where Herbage mass (kg DM/ha) = 158 x herbage height (cm) + 200, were taken randomly from each pasture strip.

Ruminal fermentation characteristics during the adaptation and intensive sampling phases were analysed using a mixed model with repeated measures over time (GenStat Release 11.1; VSN International Ltd., Hemel Hempstead, Hertfordshire, UK). The model included time of allocation (AM, PM), day of sampling (day 1, 8, 14; adaptation phase), time of sampling relative to allocation (0 to

TABLE 1: Ruminal fermentation characteristics during the adaptation and intensive sampling phases from sheep grazing a ryegrass-based pasture offered either in the morning or in the afternoon. Bolding of P values indicates significance (P <0.05).

Measurement	Adaptation				Intensive sampling			
	Morning	Afternoon	Standard error of mean	P value	Morning	Afternoon	Standard error of mean	P value
Acetate (mmol/L)	62.8	70.6	2.0	**0.03**	70.3	67.4	1.3	0.19
Propionate(mmol/L)	22.8	26.4	1.7	0.20	21.3	20.3	1.4	0.62
Butyrate(mmol/L)	12.0	14.1	0.8	0.14	11.5	11.7	0.8	0.84
Valerate(mmol/L)	0.94	1.24	0.08	0.05	0.88	0.97	0.05	0.24
Isobutyrate(mmol/L)	0.83	0.95	0.03	**0.03**	0.59	0.75	0.09	0.27
Isovalerate(mmol/L)	0.83	1.01	0.01	**0.02**	0.50	0.65	0.10	0.31
Total VFA(mmol/L)	100.2	114.3	3.9	**0.04**	105.1	101.9	2.4	0.38
Acetate/Propionate[1]	2.80	2.77	0.07	0.78	3.34	3.50	0.19	0.59
(Acetate+Butyrate)/Propionate[1]	3.34	3.32	0.20	0.96	3.88	4.09	0.22	0.54
Ammonia (μg/mL)	227.8	305.0	20.8	**0.02**	100.7	138.7	17.2	0.08
Ruminal pH	6.34	6.16	0.07	0.12	6.23	6.35	0.06	0.20

[1]Measures of non-glucogenic to glucogenic volatile fatty acids, ratios of acetate to propionate and (acetate + butyrate) to propionate.

22 hours; intensive sampling phase) and corresponding interactions as fixed effects, and animal as a random effect. Day of sampling was deemed non-significant for all variables tested, and was therefore withdrawn from the model. A spline term (Verbyla *et al*., 1999) for each animal was used to model random departures from the overall 24-hour pattern.

RESULTS

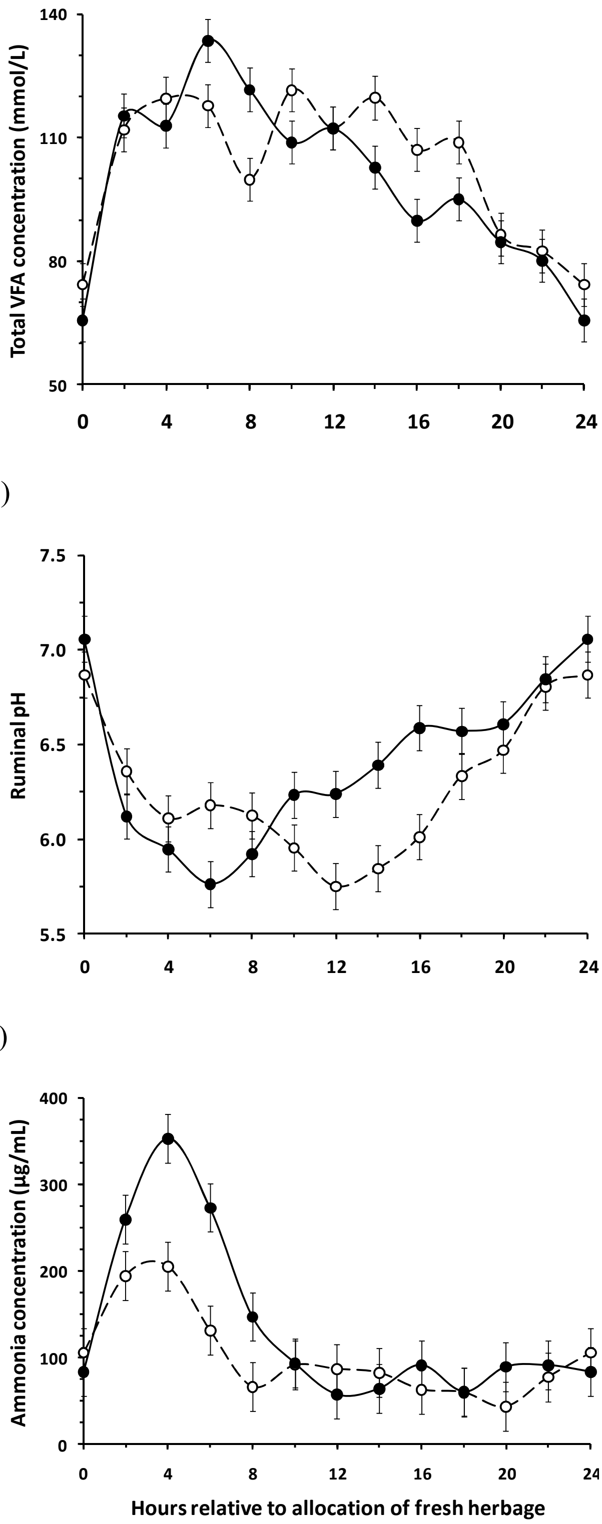
Daily DM intake, obtained from pre- and postgrazing herbage mass, was similar among dietary groups (1165 g DM/wether; 22.1 g DM/kg live weight). During the adaptation phase (Days 1, 8, and 14), acetate, isobutyrate, isovalerate, total volatile fatty acids (VFA), and ammonia concentrations were greater ($P < 0.05$) for the PM group, whereas the ratio of non-glucogenic to glucogenic VFA did not differ (Table 1). During the intensive sampling phase (Days 15 to 17), mean total VFA (103.5 mmol/L) and ammonia (119.7 µg/mL) concentrations did not differ among dietary groups, but the 24-hour pattern of time relative to allocation, varied considerably for all variables measured ($P < 0.001$). There was a significant interaction between treatment and time relative to feed allocation when the individual sampling points were analysed; total VFA concentrations from the PM group were greater at six and eight hours ($P < 0.05$) post allocation, whereas total VFA concentrations from the AM group were greater at 14 and 16 hours ($P < 0.05$) post allocation (Figure 1a). The daily pattern of ruminal pH mirrored the daily VFA pattern; ruminal digesta pH values were lesser from the PM group at six hours post allocation ($P < 0.05$), whereas lesser pH values from the AM group were shown at 12, 14, and 16 hours ($P < 0.05$) post allocation (Figure 1b). Ammonia concentrations from the PM group were greater at four ($P < 0.001$), six ($P < 0.01$) and eight hours ($P < 0.05$) post allocation of new herbage; concentrations were similar for all the other time points measured (Figure 1c).

DISCUSSION

The aim of this study was to examine the effects of afternoon versus morning allocation of spring herbage from pasture on aspects of ruminal metabolism that lead to increased utilisation of nutrients by the host animal. However, caution is necessary in the interpretation of results from this experiment. Changes in the concentration of ruminal ammonia, rather than a measure of microbial protein synthesis, was used to describe changes in ruminal N metabolism. Notwithstanding this limitation, the present experiment provides a measure of daily patterns of accumulation and disappearance of

FIGURE 1: Patterns of (a) total VFA concentration, (b) ruminal pH and (c) and ammonia concentration from sheep grazing a ryegrass-based pasture allotted either in the morning (○) or in the afternoon (●). Patterns are described relative to the time of allocation of new pasture. The error bars represents ± the standard error of the mean.

(a)



ruminal fermentation products for sheep grazing AM or PM herbage from 24-hour allocations. Expectedly, allocating PM versus AM fresh herbage from pasture results in a divergent fermentation process as a consequence of a more balanced fermentable C to N ratio offered to ruminants. This occurred to some extent during the adaptation phase, where total concentration of VFA was increased by offering PM herbage, largely driven by an increase in acetate concentration (Table 1). Volatile fatty acids produced as end products of microbial metabolism provide the host animal with a major source of metabolisable energy (Beever & Siddons, 1986). Assuming that the ruminal concentrations of VFA are a function of production and absorption, whereby a greater production rate induces greater VFA concentrations (Van Soest, 1994), these results suggest a greater extent of fermentation, supported by greater OMD values from the PM-allotted herbage during the adaptation phase (Table 2). Despite increased total VFA and acetate concentrations, numerical increases in the other major VFA of propionate and butyrate, from the PM group, led to similar non-glucogenic (acetate and butyrate) to glucogenic (propionate) ratios. Increased concentrations of the protein-derived isobutyric and isovaleric fatty acids from the metabolism of valine and leucine respectively, and ammonia from the PM group also support greater fermentation occurring during the first four to six hours of afternoon post-allocation during the adaptation phase.

In response to selectivity (Baumont *et al*., 2000) and spatial heterogeneity (Parsons & Dumont, 2003), ingestive behaviour, rather than a divergent chemical composition of the herbage, was presumably attributable for the changes in VFA and ammonia concentrations in ruminal digesta during the adaptation phase. With ruminants grazing temperate pastures, the longest and most intense grazing events occur at dusk. These events are often associated with a greater intake rate at this time of the day (Orr *et al*., 1997). In addition to most grazing activity occurring during daylight hours, up to 48% of total grazing time by sheep on perennial ryegrass has been reported to occur during the four-hour period before sunset (Penning *et al*., 1991). Associated with this, the proportion of time spent eating is reportedly greater following the allocation of a new strip of grass. Because more than 70% of the intake of herbage from pasture occurs within the first three to four and a half hours of grazing a new allocation (Trevaskis *et al*., 2004), grazing in the afternoon presumably equated to a greater proportion of herbage DM intake at a slightly greater WSC producing a greater total VFA concentration, and CP concentration producing a greater ammonia concentration (Table 2).

Except for a trend (P = 0.08) in mean ammonia concentration, the differences in ruminal fermentation characteristics lacked significance during the intensive sampling phase. Although mean values of most of the variables measured were similar, small changes in forage chemistry, intake, and to a larger extent, presumed changes in ingestive behaviour, affected the daily pattern of fermentation. A sharper rise in PM propionate and total VFA concentration, along with a sharper

TABLE 2: Chemical composition of herbage from a ryegrass-based pasture offered to sheep during the adaptation and intensive sampling phases that were offered either in the morning or in the afternoon.

Measurement	Dry matter (%)	Chemical composition (% dry matter)					
		Organic matter	Water soluble carbohydrate	Neutral detergent fibre	Acid detergent fibre	Crude protein	Organic matter digestibility
Adaptation phase (Nine samples collected from each group)							
Morning	15.2	91.0	14.4	50.0	24.7	17.4	82.3
Afternoon	16.2	91.1	15.4	49.1	24.3	19.2	84.1
P value	0.38	0.85	0.32	0.49	0.45	0.27	**0.02**
Intensive sampling phase (Three samples collected from each group)							
Morning	14.1	91.0	14.6	52.2	26.6	17.9	79.5
Afternoon	15.4	92.1	16.1	52.8	27.9	16.6	79.1
P value	0.44	0.42	0.18	0.61	0.31	0.35	0.77
Pooled sample							
Perennial Ryegrass							
Morning	13.6	90.9	13.7	53.8	26.4	16.3	78.7
Afternoon	14.1	90.8	16.0	52.5	25.8	17.4	82.7
White clover							
Morning	15.4	90.3	9.9	40.5	23.4	26.0	82.8
Afternoon	16.9	90.8	10.7	40.9	22.8	25.2	83.1

decline in pH within the first two hours of fermentation are indicative of greater fermentation activity occurring following PM allocation. It is important to note that the greater ammonia concentration reported during the adaptation phase (Table 1) was attributable to sampling in the two hours post new allocation, rather than dietary attributes. Ammonia concentration for the intensive sampling phase was the mean value of all samples collected during the 24-hour period. This is particularly important for ammonia concentrations since a sharp rise in concentration was observed during the first eight hours post allocation. This rise was considerably greater for digesta contents from the PM group. Concentrations thereafter were similar among dietary groups.

Mean ruminal ammonia concentrations were in general agreement with those summarised by Beever and Siddons (1986) from ruminants grazing ryegrass-based diets. Seemingly, ruminal ammonia concentrations throughout most of the 24-hour period appear to meet microbial growth requirements (Satter & Slyter, 1974), but a transient shortage could be occurring prior to new allocation of herbage (Figure 1c). The ammonia fermentation pattern seems to be consistent with a greater extent of CP fermentation rather than a shortage in ammonia utilization by microorganisms, occurring during the afternoon after new allocation of herbage. Ruminal losses of N can account for up to 30% of ingested N, and represent a major environmental liability; excess ammonia N in the rumen from digestion of dietary CP is the main source of N in urine (Reynolds & Kristensen, 2008). This imbalance could potentially be ameliorated by the net growth of WSC ingestion throughout the day, preferably at the expense of a net decline in N intake.

The most important change in diurnal nutritive value has been attributed to an increase in photosynthates, particularly in the upper layers of the sward (Delagarde *et al*., 2000). These changes often lead to a passive dilution of NDF and CP concentrations. Although results found in this experiment sustain this premise, minimal increases in afternoon DM and WSC concentrations of 19 and 17 g/kg DM respectively, were offset by minimal reductions in NDF, but not by CP concentrations (Table 2). As the complex link between greater WSC concentrations and animal responses continues to be explored, associated changes in herbage chemistry can also be influential in determining animal responses. Under strip grazing management, the timing of pasture allocation to grazing ruminants has been tested under a wide set of conditions, but few studies report on weather data, particularly the number of hours of sunshine during the experimental period. Herbage DM and WSC concentrations increased by 86 and 27 g/kg DM respectively, from 07:30 h to 19:30 h in a ryegrass pasture grazed by sheep; among other environmental factors, a daily mean of 6.2 hours of sunshine was able to elicit these responses (Orr *et al*., 1997). From the present data, a linear regression equation was obtained by regressing incremental WSC concentrations (PM – AM; g/kg DM) with the number of hours of sunshine per day, according to the following equation:

$$\Delta \text{ WSC (g/kg DM)} = 5.30 \ (\pm 1.2; \text{standard error}) \times \text{Hours of sunshine } (P = 0.003; r^2 = 0.69)$$

Unseasonal weather conditions in terms of only 3.9 hours of sunshine and 85 mm of rain, during this short-term trial were associated with smaller differences in WSC accumulation than expected. Based on our results, at least four hours of sunshine were needed to obtain PM – AM differences in WSC greater than 20 g per kg herbage DM. The implication of these findings on the implementation of nutritional management strategies warrants further investigation.

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