A comparison of microbial protein synthesis in beef steers fed *ad libitum* winter ryegrass or fodder beet

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Abstract

Microbial protein synthesis of steers (liveweight 286±9 kg) was compared with diets of *ad libitum* winter grass and fodder beet with 1 kg DM of lucerne silage. The experimental design was a four-by-two treatment comparison (experiment 1 and 2), using 11 day individual pen trials with total faecal and urinary collection. The trials assessed *in vivo* digestibility of diets, and diurnal variations in rumen parameters (pH volatile fatty acid, ammonia and urea concentrations) and urine and faeces production and N excretion. Microbial protein production was estimated from purine derivatives determined from total urine collection. Dry matter digestibility, voluntary dry matter intake and microbial protein supply to the steers, and the mean diurnal rumen pH, were higher in the steers fed the fodder-beet treatment than in those fed the winter-grass treatment. Fodder beet supplied almost twice as much microbial protein as winter grass despite a lower crude protein concentration of the diet. Total rumen VFA and ammonia concentrations were lower for the fodder-beet treatment than for winter grass but the rumen urea concentration was higher in the fodder-beet treatment. The observed high microbial protein supply with the fodder beet diets is likely the result of ruminal and extra-ruminal adaptations to greater nitrogen recycling in low dry matter diets of high energy density and low crude protein concentrations.

Keywords: microbial protein production; rumen function; nitrogen recycling; fodder beet; pasture-fed; *ad libitum* feeding; purine derivatives

Introduction

Fodder beet has been grown in New Zealand for over 100 years, however, the current system of *ad libitum* grazing of fodder beet *in situ* and with minimal supplementation is a novel approach developed in the last five years at Lincoln University by Gibbs (2014a). The fodder beet crop has relatively low concentrations of crude protein (CP), fibre and minerals, high water-soluble carbohydrates (WSC) concentrations and fermentable metabolisable energy (FME) (Gibbs 2011).

Historically, fodder beet has been a crop which was harvested and stored before feeding as a supplemental feed, but not used as the main dietary source (Anonymous, 2013). The use of other winter crops such as kale or swedes has resulted in liveweight gains of approximately 200 g/day in high producing beef systems (Woods et al. 1995). The current system of fodder beet feeding is to graze animals *in situ* on an *ad libitum* diet of fodder beet while supplying a supplemental fibre source such as lucerne silage or straw at approximately 1-2 kg DM/day. Over recent years, commercial scale trials have measured liveweight gains of 1.1 kg/day for 290-530 kg steers fed fodder beet for 150 d from March to September at stocking rates above 20 animals/ha, resulting in a mean liveweight production of greater than 3000 kg/ha in that period (Gibbs & Saldias, *unpublished data*). These liveweight gains are unexpected due to the low CP concentration (<14% DM) of the feed.

Due to the novelty of fodder-beet grazing systems, there has been very little research on the rumen N metabolism of fodder-beet diets. Given the apparent lack of protein to support the observed liveweight gains in cattle fed on this system, there is a need for research to determine the microbial protein supply to the ruminant on an *ad libitum* fodder-beet diet with low levels of supplementation.

The objective of this research was to establish if there was a significant difference between the microbial protein production in steers fed a diet of *ad libitum* winter pasture compared with a diet of *ad libitum* fodder beet with 1 kg DM supplementation. In addition, any differences in rumen environment between diets that may influence protein metabolism were to be quantified.

Materials and methods

Four yearling Charolais steers of mean liveweight 286±9kg were surgically inserted with rumen cannula into the dorsal sac. Two experiments were conducted to determine the effects of feeding a winter perennial ryegrass (cv 'Bealey', AgriSeeds, Christchurch) compared with feeding fodder-beet (cv 'Brigadier', Seed Force, Christchurch) on the microbial protein production of steers. In each experiment, the steers were placed in metabolism crates and a ten day digestibility trial with total faecal and urinary collection was conducted. Following the digestibility trial, the steers remained in the metabolism crates and ruminal pH was monitored *in situ* for 24 h using the method of Gibbs (2007).

Experiment one

The steers were fed *ad libitum* on a ration of fresh winter grass which was cut at 9 am each day and carried to the steers. Steers were fed after 10 am and 5 pm daily. The pasture was managed to maintain a herbage mass between 1800-4000 kg DM/ha to replicate the industry standard models of fodder-beet use in NZ beef production. The mean DM content of the pasture was 16% and the quality parameters of the pasture are shown in Table 1.

Trial one of experiment one was a digestibility trial. The steers were placed in metabolism crates at 10 am on day 1 and remained on *ad libitum* feed rations of winter grass until day 10. Feed intake was allocated to achieve 20% refusal level, measured from previous daily measurement. Duplicate representative sub-samples of feed were collected each day to determine DM concentration. Further sub-samples were obtained to determine feed quality and frozen at -20°C until freeze drying for analysis. Feed refusals were weighed for each individual animal, and oven dried at 68°C to a constant weight to obtain the DM concentration.

Total urine was collected and weighed daily and a 5% sub-sample was frozen for further analysis. Each day 500 ml of 25% H_2SO_4 was placed into each urine tray to reduce the pH below 3 and prevent volatilisation of urine ammonia. Total faeces were collected and weighed daily. After thorough mixing, duplicate representative samples for DM determination were obtained. A 10% sub-sample was obtained each day and bulked before freezing for further N analysis.

Trials two and three of experiment one were two sequential 24 h periods, the first monitoring rumen pH *in situ* using the method of Gibbs (2007), and the second collecting rumen digesta, urine and faecal samples at 2 h intervals. Rumen pH was monitored using an *in situ* rumen pH probe (Ionode IJ-44, Brisbane, Australia) placed on the floor of the ventral sac for 24 h beginning at 10 am on day 10. The pH probe was attached to a 1500 g steel paddle to ensure it stayed in place and pH was measured every 15 seconds over the period.

For 24 h from 10 am on day 11, rumen fluid and digesta samples were taken every two hours from the ventral sac to analyse for ammonia, urea, and volatile fatty acid (VFA) concentration. Rumen digesta were obtained by hand from the ventral sac region of the rumen. Rumen fluid samples were obtained by squeezing rumen digesta through two layers of cheese cloth. Urine and faeces were collected and weighed at each 2 h sampling period. A 70 ml subsample of urine was obtained and a 250 g subsample of faeces was obtained. For faeces, duplicate DM samples were obtained and oven dried at 68°C until a constant weight was reached. The pH of rumen fluid, rumen digesta, urine and faeces was measured at each collection. Rumen fluid for ammonia analysis and urine samples were acidified with 1 ml of 6M H_2SO_4 to inhibit ammonia volatilisation.

Experiment two

At the completion of experiment one, the steers were transitioned onto fodder beet over 14 d (Gibbs 2014b). Lucerne silage allowance was decreased over this period to a final allocation of 1 kg DM daily. After transition, the steers were observed to be on *ad libitum* fodder-beet intake for two weeks prior to the commencement of experiment two.

At the beginning of experiment two, the steers were placed into metabolism crates. Steers were fed *ad libitum* on a ration of fresh fodder beet which was collected by hand and carried to the steers daily, along with 1 kg DM lucerne silage at 10 am daily. Fodder beet was cut to separate leaf and bulb, and the leaf-to-bulb ratio fed to the steers was 1:4. This ratio was calculated from five years of previous 'Brigadier' crop assessments by this research group.

The crop yield was assessed at 23-26 tonne DM/ha at maturity. The dry matter concentration of the fodder beet leaf was 14.3% and the bulb 10.2%, and the proximate analyses of all feed components are shown in Table 1.

Trial one of experiment two was a digestibility trial. The steers remained on *ad libitum* fodder beet. Duplicate representative sub-samples of all feeds were collected and dried each day to obtain DM concentrations of the feed. Further sub-samples were obtained and kept frozen at -20°C for later analysis. Feed refusals were collected, weighed, and oven-dried to obtain the DM concentrations. Urine and faecal collection and sub-sampling was carried out as described in experiment one.

Trials two and three of experiment two involving the *in situ* rumen pH measurements and the rumen digesta, urine and faecal sampling were carried out as described in experiment one.

On each day of the trial duplicate samples of winter grass, fodder beet, lucerne silage and faeces were weighed and dried at 68°C to a constant weight to determine dry matter %.

Urine samples were thawed under refrigeration at a temperature of 4°C, and subsampled (100 ml). These subsamples were analysed for purine derivatives, ammonia, urea and total N concentrations. Analysis of purine derivative concentration of urine was carried out according to the method of Chen & Gomes (1992). Ammonia was analysed using an RX Daytona analyser (based on the method of Cray (2013)), and total N was measured using the elemental analyser Vario Max CN (based on the Dumas combustion method of Etheridge (1998)).

Bulked faeces were stored at -20°C then thawed under refrigeration at 4°C, subsampled and freeze dried, then analysed for total N as described above.

Dry matter digestibility of each steer across the nine day period of each experiment was determined using the relationship between total DM intake and faecal dry matter output.

To calculate the diurnal pattern for pH, the pH measurements recorded every 15 seconds were averaged for successive 10 minute periods for each diet treatment for the 24 h period, resulting in 160 pH records (four per minute for 10 minutes for four steers) (Gibbs 2007). 'Bout counts' were calculated using the mean recorded diurnal pH measurements from each steer in the winter grass and the fodder-beet diet treatments (Gibbs 2007). If the recorded pH was less than each of three thresholds for two minutes, it was recorded as a single bout for that threshold and the duration (in minutes) of that bout was calculated. The means for the bout counts and bout times were then calculated for the steers in each diet treatment.

Microbial crude protein production (MCP) was estimated using the daily purine derivative (PD) mass in urine from each steer via the method of Chen & Gomes (1992). Mean daily urine volume for each steer was used and PD concentrations of the subsampled bulked urines to calculate the daily mass of PD excreted in the urine of each steer. This was used in Equation 1 of Chen (1998) to calculate the amount of microbial purines absorbed daily:

Equation 1: $Y = 0.85X + (0.385 \text{ x liveweight}^{0.75})$

Where Y = the daily PD excretion and X = the daily purine derivative absorption.

Once X is determined, the daily microbial N yield as calculated by Equation 2 of Chen (1998):

Equation 2: Microbial N (g N/d) = ($\boldsymbol{X}$ (mmol/d) x 70)/0.116 x 0.83 x 1000 = 0.727$\boldsymbol{X}$

Statistical analysis was carried out using GenStat 16 (Hampshire, UK). Analysis of variance (ANOVA) was carried out using feed (winter grass vs fodder beet) as the treatment and animal as the block structure. Least significance difference (LSD) test (P=0.05) was carried out to compare means with significant differences demonstrated from ANOVA.

A t-test was performed to test for significance in the diurnal pattern of rumen digesta pH between the winter-grass and the fodder-beet diet treatments.

Results

Feed composition

The proximate analyses of the feeds used in these trials are displayed in Table 1.

Table 1 Feed composition of winter grass, lucerne, fodder-beet bulb and fodder-beet leaf fed to steers.

%	Winter Grass	Lucerne	Fodder Beet	
			Leaf	Bulb
DM	16.3	59.2	14.3	10.2
OM	88.6	88.3	80.0	91.4
CP	16.3	20.6	17.5	11.9
NDF	39.1	39.5	29.2	12.3

Values of organic matter (OM), crude protein (CP), and neutral detergent fibre (NDF) are expressed as percentage (%) of dry matter (DM).

In vivo digestibility

The *in vivo* digestibility of dry matter, organic matter and digestible organic matter for the winter grass and the fodder-beet diet treatments are displayed in Table 2, with the voluntary daily DMI. Fodder beet was significantly higher (P<0.05) in DMD% and DMI compared with winter-grass diet.

Table 2 *In vivo* dry matter digestibility (DMD), organic matter digestibility (OMD), digestible organic matter digestibility (DOMD) in % of dry matter, and daily dry matter intake (DMI) in kilograms per day, of the winter grass and fodder beet treatments.

	DMD%	OMD%	DOMD%	DMI (kg/d)
Winter Grass	76.2	83.4	73.9	4.5
Fodder Beet	78.5	83.8	75.2	6.6
SEM	0.007	0.004	0.005	0.19
LSD	0.32	0.018	0.022	0.84
P Value	0.032	0.076	0.056	0.004

Microbial protein production and efficiency

The estimated daily post-rumen microbial protein supply in g N and in g N/kg DMI to the intestines in both diet treatment groups is displayed in Table 3. There was a highly significant difference (P<0.01) between diet treatments in mean g microbial N /d, with the fodder-beet treatment observed to provide greater supply, and a less significant (P<0.05) difference between treatments in mean g microbial N/ kg DMI. Mean daily urine production (kg/d) was significantly lower (P<0.05) in the winter-grass treatment (14.2) compared with the fodder-beet treatment (29.7).

Table 3 Microbial nitrogen production (g N) and microbial nitrogen production per kg DMI (g N/kg DMI) for individual steers for the winter grass and fodder beet treatments.

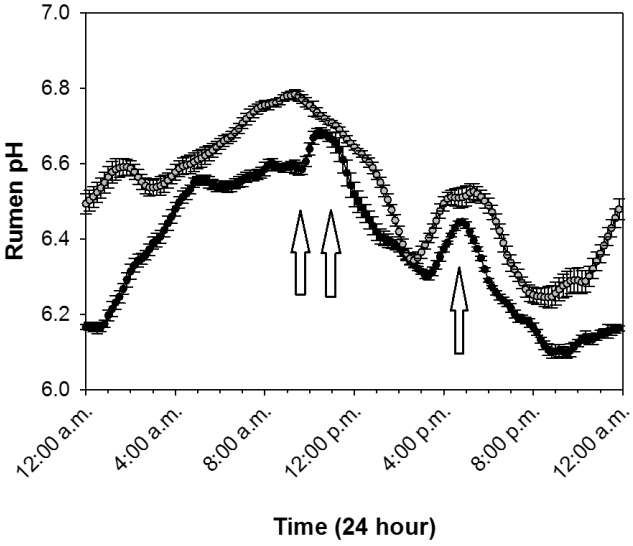
Treatment	Steer	Microbial Nitrogen (g)	Microbial N efficiency (g N/kg DMI)
Winter Grass	Mean	56.4*	12.5#
	SEM	5.21	1.05
Fodder Beet	Mean	104.7*	15.5#
	SEM	6.20	0.51

The standard error of the mean (SEM) is displayed for each, and * indicates a significant difference between treatment means of <0.01, while # indicates a value of <0.05.

Diurnal pattern of rumen digesta pH

The diurnal pattern of mean recorded rumen digesta pH for winter-grass and fodder-beet diets is displayed in Figure 1. The pH recorded was higher for the fodder-beet treatment at most intervals, with a significant difference (P<0.001) in digesta pH between the two diet treatments, and a similar trend to 6 h post-prandial reduction from 2 h post-prandially in both treatments, which persisted for approximately 16 h. There were highly significant differences (P<0.001) between the treatments for each 8 h time interval (10 am-6 pm, 6 pm-2 am, 2 am-10 am). The fodder-beet treatment was observed to have very few recorded pH values below 5.8, largely maintaining pH in the range above 6.0 (Table 4, Figure 1). In both treatments decreased pH values occurred more commonly beyond 6 h post-prandially.

Figure 1 The diurnal pattern of the means (for every ten minutes of twenty four hours) of rumen digesta pH (±SEM) recorded from the ventral sac by indwelling sensors for four steers fed either ad libitum winter-grass or fodder-beet diets. The arrows (left to right) represent feeding of the lucerne supplement, the first half of the fodder-beet ration, and the second half of the fodder-beet ration, respectively. The winter grass was fed at 10 am daily and 5 pm.



The mean number and duration of 'bouts' for the diurnal pattern of digesta pH for the winter-grass and the fodder-beet diet treatments are displayed in Table 4. The winter-grass diet treatment was observed to have an increased number and duration of bouts under 6.0, and a fivefold increase in mean bout numbers under 5.8. However, in all thresholds the fodder-beet treatment bout duration was longer, and particularly at lower thresholds.

Rumen VFA, ammonia and urea concentrations

The diurnal pattern for the total VFA, ammonia and urea concentration in rumen fluid obtained from the ventral sac for the winter-grass and fodder-beet diet treatments are displayed in Figure 2(a-c). The winter-grass diet treatment had a higher mean VFA concentration, and a significantly higher ($P<0.001$) VFA concentration at the 8 pm-10 am sampling period. Rumen ammonia was significantly ($P<0.01$) lower in the fodder-beet treatment across the diurnal period, and rumen urea concentration significantly ($P<0.01$) higher.

Figure 2a-c Diurnal pattern of total rumen volatile fatty acid (VFA) concentration (mmol/l), ammonia (mg/L) and urea (mmol/L) of steers fed winter grass (●) and fodder beet (○). Values are means ± SEM.

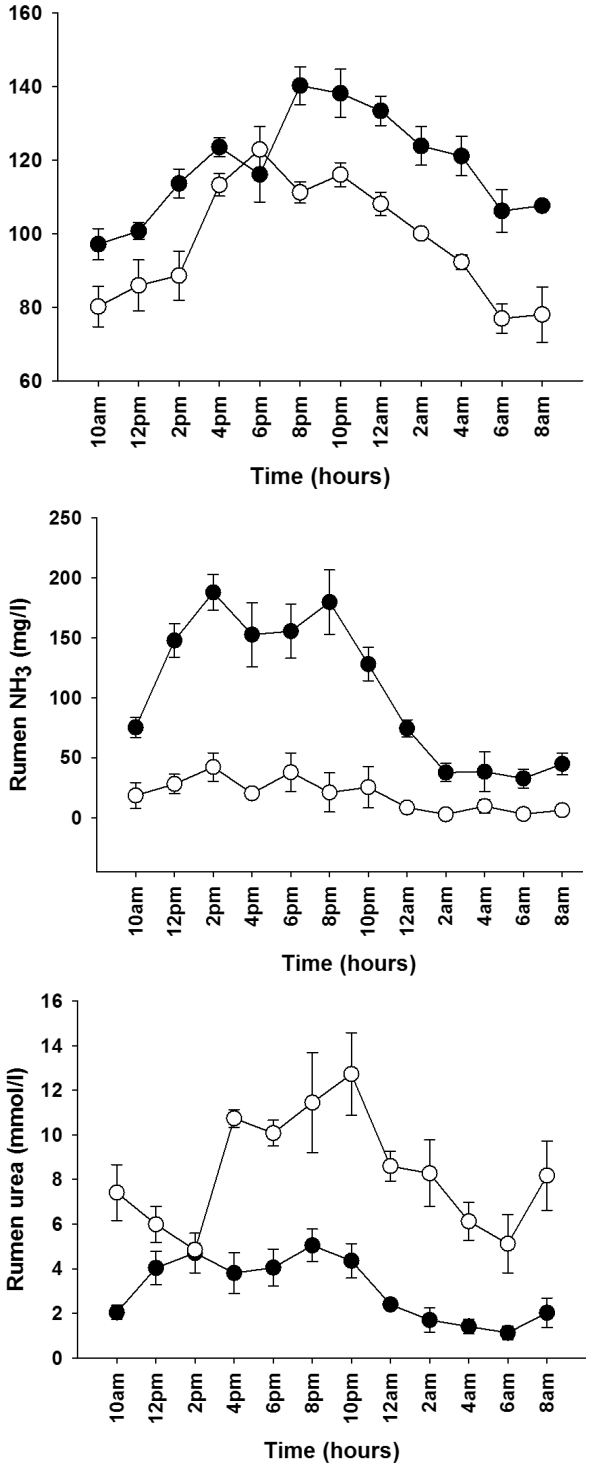


Table 4. The mean number and duration in minutes (mins) of bouts of pH below three thresholds (<5.8, <6.0, <6.4) calculated from the diurnal pH measurements taken every fifteen seconds for twenty four hours by indwelling sensors in the rumen ventral sac, for the winter-grass (grass) and the fodder-beet (FB) diet treatments. Standard errors (SEM) of mean durations in minutes are included.

	Bouts <5.8:			Bouts <6.0:			Bouts <6.4:		
Feed:	Mean bouts per day:	Mean mins per bout:	Mins per bout SEM:	Mean bouts per day:	Mean mins per bout:	Mins per bout SEM:	Mean bouts per day:	Mean mins per bout:	Mins per bout SEM:
FB	0.24	148.5	0	0.97	59	45.913	6.09	71.57	35.789
Grass	1.23	5.05	1.062	2.46	35.23	10.346	8.13	86.52	23.746

Discussion

This is the first study comparing microbial protein production of fodder beet and pasture, and the first report of a significantly increased microbial protein production with *ad libitum* fodder-beet feeding compared with winter pasture. The fodder-beet diet resulted in a significantly higher microbial protein production than the winter-grass diet despite the lower CP concentration of the fodder-beet diet treatment. In addition, the rumen environment between treatments was significantly different, with fodder beet observed to have higher rumen pH (Figure 1) and ammonia, but lower total VFA and ammonia concentrations (Figure 2a-c).

Microbial protein efficiency (g N/kg DMI) was also observed to be significantly higher on the fodder-beet diet. The twofold increase in urine from the steers on the fodder-beet diet demonstrates the increased water loading of the rumen in that treatment, due to the low DM content of the feed and the high voluntary DMI. As previous literature suggests, an increase in passage rate will increase the efficiency of microbial protein production due to lower cell lysis and microbial N recycling in the rumen (Dewhurst et al. 2000). However, as the rumen fluid passage rates were not significantly different between the treatments (data not presented), this suggests that the increased dietary water was leaving the rumen at an increased rate via the rumen epithelial route. This is the first reported observation of this in winter-crop fed cattle, and if subsequently demonstrated to be repeatable, it suggests there may be substantial physiological adaptations beyond the rumen to grazing fodder beet to maintain high DMI and subsequent production.

There is indirect evidence for this in the lower total VFA concentrations and higher rumen pH observed in the fodder-beet treatment (Figure 1 and 2a-c), despite the very high ME intake and very rapid, early DM disappearance of the bulb in the rumen that indicates high FME release (data not presented). The mechanism for increasing VFA absorption is greater epithelial blood flow (Storm et al. 2012), and this will also increase water removal, and potentially, urea recycling.

The increases in DMI per kg live weight observed were threefold greater (Table 1) with the fodder-beet diet than any live weight increase would predict, and therefore in this study voluntary DMI/kg live weight (kg DMI/kg LWT) was 36.1% higher for the fodder-beet treatments. The magnitude of the increase in mean daily microbial protein production (84.5%: Table 3) for the fodder-beet diet was similarly increased. This suggests that some characteristics of the fodder-beet diet were factors in the increased microbial protein supply, as opposed to simply increased DMI. Given it is not greater rumen fluid passage rates, it would appear to be associated with the greater rumen efficiency of N use (Table 3). The unusually high urea concentrations in the rumen relative to the ammonia may support for this, and suggest changes in N recycling to the rumen are centrally involved.

It follows that when MCP supply (g) is expressed against DMI (12.5 vs. 15.5g N /kg DMI for winter grass and fodder beet respectively), this efficiency of MCP supply is significantly different between the two treatments. The high supply of FME in both diets supports high microbial growth rates and suggests that microbes will not be limited by energy supply. Therefore, it would appear that there are other reasons for the increased rumen microbial N efficiency in the fodder-beet treatment, and perhaps the most likely explanation involves N recycling, and the relative abundances of rumen ammonia and urea observed in this study.

Rumen pH is primarily modulated by the removal of acid from the rumen, by either absorption through the rumen wall or by way of passage through the gastrointestinal tract (Dijkstra, 1994). In the current study the winter-grass treatment had a lower ME intake than the fodder beet treatment, but a lower rumen pH. This is the first reported observation of a relatively high rumen pH on an *ad libitum* fodder-beet diet. Previous work by this research group (Gibbs, *unpublished data*) observed a rapid decrease in post-prandial pH of steers fed fodder beet at restricted intakes, which encouraged rapid consumption, with rumen pH values recorded as low as 5.0-5.2, a very different pattern to that seen in the present study. This demonstrates the diurnal pH pattern and the rumen environment is influenced by both intake and grazing behaviour, as a result of restricted feeding, and this is consistent with reported drivers of intake patterns and subsequent animal physiology shifts (Baumont et al. 2006; Gregorini et al. 2009; Leaver et al. 1969).

Given the multifaceted control of grazing intakes, this difference between restricted and *ad libitum* fodder-beet diets suggests that at least some adaptations to the latter by cattle may involve extra-ruminal mechanisms – grazing behaviour pattern shifts, for example. Despite the extra-ruminal mechanisms that may be operating in *ad libitum* fodder-beet diets, one effect of the flattening of intake across the diurnal cycle would be to encourage a rumen environment of higher pH and less diurnal variation that is conducive to microbial protein production. This may be a factor in the higher efficiency of microbial protein production observed in this study.

Conclusions

This study has demonstrated that *ad libitum* diets of winter grass or fodder beet had significant differences in the supply of microbial protein to beef steers. Voluntary DMI observed on the fodder-beet diet was higher than that of winter grass, and resulted in an increase in N intake, but neither explained fully the increased efficiency observed. The diurnal pattern of rumen pH was higher on the fodder-beet diet, the VFA and ammonia concentrations were lower, enough to be considered an impediment to normal rumen microbial function. However, there was no evidence of this reduced function, and the increased urea and reduced ammonia concentration in the rumen may be indicators

of a specific strategy of adaptation. The implications of high efficiency of N to microbial protein transformation in certain diets means further investigation of this area of N metabolism is warranted.

These findings may explain the animal production results that have been observed in the form of high daily liveweight gains on a commercial scale. These results show that on an *ad libitum* fodder-beet diet, despite the low CP of the feed, supplies higher microbial protein than winter grass and that supply is adequate for high growth rates (>1 kg/ d) in steers.

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