Intake, retention time in the rumen and microbial protein production of *Bos indicus* steers consuming grasses varying in crude protein content

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Abstract. Feed intake, rumen function, microbial protein (MCP) production and the efficiency of MCP production were determined in steers fed four different forage hays varying markedly in crude protein content. Low quality tropical forage (speargrass and Mitchell grass) hays had lower crude protein content, higher neutral detergent fibre content and lower digestibility than a medium quality tropical forage (pangola grass) hay and a temperate forage (ryegrass) hay. Steers fed speargrass and Mitchell grass hays had lower MCP production (80 and 170 g MCP/day, respectively) and efficiency of MCP production [78 and 79 g MCP/kg digestible organic matter (DOM), respectively] than steers fed pangola grass (328 g MCP/day; 102 g MCP/kg DOM) and ryegrass (627 g MCP/day; 135 g MCP/kg DOM) hays, which was directly related to the supply of DOM and rumen degradable protein. Intake was greatest for ryegrass hay, followed by pangola grass, Mitchell grass and speargrass hays [17.6, 15.6, 10.1 and 5.5 g DM/kg W.day, respectively]. The retention time of DM in the rumen was 72.1, 47.7, 28.6 and 19.1 h for speargrass, Mitchell grass, pangola grass and ryegrass hays, respectively, with a similar trend apparent for the retention time of neutral detergent fibre, lignin, chromium-EDTA and ytterbium labelled digesta. The difference in the protein : energy ratio of absorbed substrates (measured as efficiency of MCP production) did not appear to account for all the differences in intake, nor did a purely physical mechanism.

Introduction

Low microbial crude protein (MCP) production and low efficiency of MCP production [EMCP; g MCP/kg digestible organic matter (DOM)] in cattle consuming tropical pastures are most likely associated with inadequate rumen degradable protein (RDP) supply and a low turnover rate or long retention time of digesta in the rumen, restricting microbial growth and EMCP (Thornton and Minson 1973; Poppi *et al.* 1981). Long retention time and low RDP are largely related to the physical properties and anatomical structure of forages, and there is little information on variation in EMCP of tropical forages and hence the variability in protein : energy (P : E) of absorbed substrates. It is hypothesised that a difference in EMCP between forages would alter the P : E of absorbed substrates and, from Egan's hypothesis (Egan 1977), result in different intake. The P : E of absorbed substrates from tropical forages is not known, but EMCP provides a measure of this as MCP provides the bulk of protein absorbed by cattle consuming tropical forages.

The objective of this experiment was to study intake, retention time of digesta in the rumen and MCP production in steers consuming tropical forages varying in crude protein (CP) content. Ryegrass was also examined as this represented high CP temperate forage.

Materials and methods

All procedures were approved by the University of Queensland Animal Ethics Committee.

Animals, experimental design, diets and feeding

The experiment was carried out at the University of Queensland's Mt Cotton Research Farm, Brisbane, Australia. Eight rumen-cannulated 3/4 Brahman crossbred steers (424 ± 37 kg) were weighed before the commencement of the experiment and randomly allocated to treatments. The experimental design consisted of two 4 by 4 Latin squares, with four treatments (four forages) carried out concurrently from October to March. Each experimental period was carried out over 21 days, consisting of a 14-day preliminary feeding period, comprising of 10 days in pens and 4 days in metabolism crates, followed by a 7-day collection period in metabolism crates. Between runs, steers grazed on pangola pasture (*Digitaria eriantha*) for 2 weeks with mineral blocks containing N, Ca, P, S, Cu, Co, Fe and Mg (Go-Block, Olsson's Pty Ltd, Australia). The treatments used in the present study were low CP tropical grass hays, speargrass (*Heteropogon contortus*; Brian Pastures, Gayndah, Qld, Australia) and Mitchell grass (*Astrebla* spp.; Barkly Tableland,

NT, Australia); a medium CP tropical grass hay, pangola grass (Mt Cotton, Qld, Australia), and a high CP temperate grass hay, ryegrass (*Lolium multiflorum*. cv. Aristocrat; Mt Cotton) (Table 1). All hays were chopped to less than 10 cm in length before feeding. Steers were fed once daily at 0800 hours in the pens and equal amounts were offered at hourly intervals by an automatic feeder in the metabolism crates. For the first 12 days of the preliminary period, the animals were offered hay *ad libitum* (previous day's intake plus 20%) to determine average daily feed intake. For the final 2 days of the preliminary period and the 7-day collection period, feed was offered at a constant level of 110% of average feed intake over the first 12 days of the preliminary period.

Sampling procedures and measurements

Feed intake was determined daily. Total faecal and urine outputs were collected daily during the collection period. Urine was collected into trays with dilute sulfuric acid (0.9 M) so as to keep pH below 3. Rumen fluid was collected to determine NH_3-N. Plasma was obtained from blood samples collected from the tail vein of each steer. Retention times of chromium-EDTA (Cr-EDTA) and ytterbium (Yb) chloride ($YbCl_3.6H_2O$) markers were measured on Day 3 of the collection period. A single dose of Cr-EDTA solution (~154 mg Cr/100 kg W), was administered across four different sites in the rumen. At the same time, 10 g of Yb-labelled hay (2 g Yb) was evenly distributed throughout the rumen. Rumen fluid and rumen digesta samples were taken before dosing (0 h) and 3, 6, 9, 12, 24 and 32 h after dosing. Retention time and digesta load of each feed type in the rumen were measured as in Poppi *et al*. (1981).

Analytical techniques

Subsamples of feed offered, feed residues and faeces were dried to a constant weight at 60°C in a fan forced oven to measure DM and combusted in a muffle furnace at 550°C for ~4.5 h to measure organic matter (OM). Ash free neutral detergent fibre (NDF) and lignin were measured in an Ankom fibre analyser (ANKOM 220; Ankom Technologies, Macedon, NY, USA). Total N content was measured using the Leco system (LECO FP-428; LECO Corporation, St Joseph, MI, USA). Ammonia-N concentration in rumen fluid was measured by distillation. Plasma urea-N concentration was determined spectrophotometrically using a BUN reagent (Trace Scientific; Noble Park, Vic., Australia). Chromium in rumen fluid was analysed after centrifugation. Ytterbium was analysed in dried, ground whole digesta using the method of de Vega and Poppi (1997). All samples were

analysed for Cr-EDTA and Yb by inductively coupled plasma atomic emission spectrometry. Purine metabolites in urine samples were determined by a modified procedure of Balcells *et al*. (1992) by HPLC (Agilent 1100 Series; Forest Hill, Vic., Australia). Estimation of MCP production was as in Bowen *et al*. (2006).

Statistical analysis

The statistical significance of treatment effects on each variable was tested by analysis of variance (ANOVA), with terms for hay type (treatment), steer and run. The significant pairwise differences between hay types were tested using the protected least significant difference (l.s.d.) procedure, if the ANOVA test of the hay type effect was significant. All data were analysed using the statistical package GENSTAT 2007 (GENSTAT for windows, 10th edn, VSN International, Oxford, UK).

Results

Chemical composition of the hays

The nutrient composition of the hays varied markedly (Table 1). The ryegrass hay had the highest proportion of leaf (94%), followed by pangola grass (64%), Mitchell grass (63%) and speargrass (49%). For the tropical grasses, these values were not reflected in NDF content but had some association with lignin content. Nitrate level of ryegrass was high at 11 g/kg DM.

Intake, digestibility and retention time

Dry matter intake of ryegrass and pangola grass hays was similar and both were greater than speargrass and Mitchell grass hays (Table 2). The DM digestibility of ryegrass hay was greater than that of pangola grass and speargrass hays, which were in turn greater than Mitchell grass hay. Total DOM intake (DOMI) decreased significantly from ryegrass, pangola grass, Mitchell grass to speargrass. Retention time of DM, NDF, lignin, Cr-EDTA and Yb were much longer in the rumen of steers fed speargrass and Mitchell grass hays than pangola grass and ryegrass hays (Table 2). All steers had a similar DM rumen digesta load, but animals fed speargrass had a much lower total digesta load (Table 2).

Microbial protein production and rumen parameters

Microbial protein production was greatest in steers fed ryegrass hay, which was twofold, 3.6-fold and 7.8-fold greater than MCP production in steers fed pangola grass, Mitchell grass and speargrass hays, respectively (Table 3). The EMCP between

Table 1.   Nutrient composition of speargrass, Mitchell grass, pangola grass and ryegrass hays
NDF, neutral detergent fibre

Parameter	Speargrass	Mitchell grass	Pangola grass	Ryegrass
Dry matter (g/kg)	910	917	893	877
Organic matter (g/kg DM)	921	907	934	889
Crude protein (g/kg DM)	25.7	29.7	75.5	199.8
Neutral detergent fibre (g/kg DM)	709	692	691	584
Lignin (g/kg DM)	71.8	42.5	66.2	24.6
Lignin (g/kg NDF)	101.3	61.4	95.8	42.1

Table 2.   Intake, digestibility and retention time of grass and hay fed to steers *ad libitum* at hourly intervals
Values are means and standard error of the difference of the means (s.e.d.). Within rows means with different lowercase letters are significantly different ($P < 0.05$). Cr-EDTA, chromium-EDTA; DOM, digestible organic matter; NDF, neutral detergent fibre; n.d., too low to be detected accurately; OM, organic matter; RT, retention time; Yb, ytterbium

Parameter	Speargrass	Mitchell grass	Pangola grass	Ryegrass	s.e.d.
		Intake			
DM (g/kg W.day)	5.5a	10.1b	15.6c	17.6c	1.36
DOM (g/kg W.day)	2.4a	4.5b	7.9c	11.4d	0.60
		Digestibility			
DM (%)	46.5ab	40.7a	54.6b	69.7c	3.79
OM (%)	50.4a	46.2a	54.7a	71.0b	3.98
NDF (%)	54.8a	50.8a	56.8a	79.7b	3.72
		Retention time and digesta weight in rumen			
Rumen digesta total weight (g/kg W)	119a	144ab	170b	154b	13.8
Rumen digesta DM weight (g/kg W)	19	20	18	14	2.0
RT DM (h)	72.1a	47.7b	28.6c	19.1c	4.59
RT NDF (h)	69.8a	47.8b	29.1c	20.7c	4.77
RT lignin (h)	129.6a	120.5a	53.4b	n.d.	9.19
RT Cr-EDTA (h)	33.7a	31.7b	13.7b	10.2b	2.94
RT Yb (h)	57.5a	41.6ab	28.9b	13.2c	4.94

Table 3.   Microbial protein production and rumen parameters of grass and hay fed to steers *ad libitum* at hourly intervals
Values are means and standard error of the difference of the means (s.e.d.). Within rows means with different lowercase letters are significantly different. CP, crude protein; DOM, digestible organic matter; EMCP, efficiency of microbial protein production; MCP, microbial protein; RDP, rumen degradable protein

Parameter	Speargrass	Mitchell grass	Pangola grass	Ryegrass	s.e.d.
MCP (g/kg W.day)	0.2a	0.4b	0.8c	1.5d	0.07
EMCP[A] (g MCP/kg DOM)	107.7	110.2	102.3	135.0	23.14
EMCP[B] (g MCP/kg DOM)	77.7a	78.6a	102.3b	135.0c	6.77
CP : DOM (g/kg)	56.7	64.1	137.4	285.4	
RDP : DOM[C] (g/kg)	42.6	48.1	103.1	214.1	
Plasma urea-N (mg/dL)	16.0a	9.4a	11.1a	48.0b	3.43
Rumen NH$_3$-N (mg N/L)	48.5ab	31.3a	57.1b	191.0c	10.70

[A]All data included.
[B]Two outlying data points not included due to extremely low hay intakes.
[C]Assumes a CP degradability of 75% (McLennan *et al.* 1997).

forage types was only significantly different when two outlying data points were removed from the analysis. The two outlying data points removed were for one animal fed speargrass and one animal fed Mitchell grass, which had very low intakes over the duration of the experiment, thereby inflating the calculation of EMCP. In all other analyses, the data from these animals were used as they were consistent over the duration of the experiment.

The plasma urea-N concentration was higher in steers fed ryegrass hay compared with steers fed the three tropical forage hays, with no differences observed between the tropical forages. Rumen NH$_3$-N concentration of steers fed ryegrass hay was greater than those fed pangola grass hay, which in turn was higher than steers fed Mitchell grass hay and speargrass hay.

Discussion

The four forages used in the present study differed significantly in CP content, intake, digestibility, retention time and MCP production, and as such they provided a good model to study MCP production and its role in intake regulation in low CP forages. MCP production varied markedly between diets and was highest in animals consuming ryegrass hay, followed by pangola grass, Mitchell grass and speargrass hays, largely affected by DOM intake. The EMCP values for the tropical forages were below that suggested by Freer *et al.* (2007) of 130 g MCP/kg DOM, when N supply is adequate, and were directly related to RDP supply, which was inadequate for all three tropical forages (Table 3). The long retention time of OM, NDF and Cr-EDTA would also affect microbial growth and turnover within the rumen. The gain of MCP across the rumen with respect to RDP supply for the CP deficient forages indicates the role of recycling of N. In contrast, ryegrass hay showed a marked loss of N across the rumen in agreement with other studies (Cruickshank *et al.* 1992). In general, these values support the concept that RDP is the main limitation to MCP production in tropical forages.

The P : E of absorbed substrates, as measured by EMCP, did not explain the difference in intake between two CP deficient forages, nor did a physical mechanism based on rumen fill and retention time. The EMCP was related directly to the supply of RDP in all three tropical forages. Differences in intake between forages have previously been related to the physical regulation of intake, where rumen fill is at a constant high level and intake is directly related to the retention time of digesta in the rumen (Thornton and Minson 1973; Poppi *et al.* 1981). However, Egan (1977) suggested that when animals are fed low CP diets, their intake is below this potential physical level because the P : E of absorbed substrates is low and there is a metabolic mechanism for intake regulation. Since MCP production is the major source of metabolisable protein from low CP diets, it follows that MCP production and EMCP are the major factors affecting the P : E of absorbed substrates. Weston (1996) proposed that there was an interaction between metabolic, physical and other factors controlling intake, and that rumen load might vary with the energy deficit of the diet, increasing in load up to a maximum level as the energy deficit increased. Cattle consuming speargrass had the lowest intake and the lowest level of total rumen load (but similar DM load) and the highest energy deficit. Metabolisable energy intake as a proportion of maintenance metabolisable energy was 0.35, 0.55, 1.05 and 1.67 for speargrass, Mitchell grass, pangola grass and ryegrass, respectively, indicating the large energy deficit on the two low CP forages with speargrass having by far the greater energy deficit. Visually, the rumens of animals consuming speargrass were not full, with plenty of space in the dorsal sac, whereas the rumens of animals fed the other tropical forages were visibly full and tightly packed well into the dorsal sac. The level of fill might be compared with data of Poppi *et al*. (1981), where cattle had tightly packed rumens under a N supplement regime (total rumen load was 165–197 g/kg W and total rumen DM load was 17.9–23.6 g DM/kg W). With the data from speargrass, Weston (1996) suggests that other factors relating to palatability and other sensory cues override the simple metabolic or physical mechanisms. Nevertheless, Hunter and Siebert (1987) have shown the marked intake response to extra supply of absorbed amino acids with speargrass, which suggests that this is a major factor. This experiment was designed primarily to examine the MCP production and EMCP of tropical forages varying in CP content, and to examine the association of P : E with intake especially with the low CP forages. Within the constraints of this experiment, it might be concluded that the P : E derived from EMCP was not a factor controlling intake of the two low CP forages. These same parameters need to be examined with and without a protein supplement for the factors controlling intake of these low CP diets to be more clearly defined. In the present experiment, intake and DOMI of the forages varied widely. The intake of ryegrass hay was not as high as expected (Cruickshank *et al.* 1992), but this might have been associated with the high nitrate levels measured at 11 g/kg DM.

One quantitative outcome, irrespective of the intake mechanism, is the very long retention time of digesta DM, NDF, lignin and water (Cr-EDTA) or digesta (Yb) markers in the rumen of steers consuming speargrass and Mitchell grass compared with pangola grass and ryegrass (Table 2), which has implications for EMCP, where dilution rate is a key factor

controlling microbial growth (Dijkstra *et al.* 1998). These retention time values with low CP forages were much longer than found by Poppi *et al.* (1981).

In the present study, rumen NH_3-N and plasma urea-N increased with increasing CP content of the forage. Rumen NH_3-N concentration in the rumen of steers fed the Mitchell grass and speargrass hays was lower than the minimum level (50 mg NH_3-N/L) suggested for optimal rumen function (Satter and Slyter 1974). However the differences between the values did not reflect the magnitude of the differences in MCP production.

In conclusion, MCP production in steers consuming tropical forages was directly related to RDP supply. The P : E of absorbed substrates as measured by EMCP was not associated with the difference in intake of the low CP forages. There did not appear to be a consistent physical or metabolic mechanism regulating intake of the three tropical forages.

Acknowledgements

References

Balcells J, Parker DS, Guada JA, Piero JM (1992) Simultaneous analysis of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. *Journal of Chromatography Biomedical Applications* **575**, 153–157. doi:10.1016/0378-4347(92)80517-T

Bowen M, Poppi DP, McLennan SR, Doogan VJ (2006) A comparison of the excretion rate of endogenous purine derivatives in the urine of *Bos indicus* and *Bos taurus* steers. *Australian Journal of Agricultural Research* **57**, 173–177. doi:10.1071/AR05182

Cruickshank GJ, Poppi DP, Sykes AR (1992) The intake, digestion and protein degradation of grazed herbage by early weaned lambs *The British Journal of Nutrition* **68**, 349–364. doi:10.1079/BJN19920094

de Vega A, Poppi DP (1997) Extent of digestion and rumen condition as factors affecting passage of liquid and digesta particles in sheep. *The Journal of Agricultural Science* **128**, 207–215. doi:10.1017/S0021859696004078

Dijkstra J, France J, Davies DR (1998) Different mathematical approaches to estimating microbial protein supply in ruminants. *Journal of Dairy Science* **81**, 3370–3384.

Egan AR (1977) Nutritional status and intake regulation in sheep. VIII. Relationship between the voluntary intake of herbage by sheep and the protein/energy ratio in the digestion product. *Australian Journal of Agricultural Research* **28**, 907–915. doi:10.1071/AR9770907

Freer M, Dove H, Nolan J (Eds) (2007) 'Nutrient requirements of domesticated ruminants.' (CSIRO Publishing: Melbourne)

Hunter RA, Siebert BD (1987) The effect of supplements of rumen-degradable protein and formaldehyde-treated casein on the intake of low-nitrogen roughages by *Bos taurus and Bos indicus* steers at different stages of maturity. *Australian Journal of Agricultural Research* **38**, 209–218. doi:10.1071/AR9870209

McLennan SR, Kidd JF, Hendrickson RE, Jeffrey M, Poppi DP, Martin PM (1997) Seasonal changes in the degradability of protein in a tropical grass pasture. In 'Recent advances in animal nutrition in Australia'. (Eds JL Corbett, M Choct, JV Nolan, JB Rowe) p. 252. (University of New England: Armidale)

Poppi DP, Minson DJ, Ternouth JH (1981) Studies of cattle and sheep eating leaf and stem fraction of grasses. I. The voluntary intake, digestibility and retention time in the reticulo-rumen. *Australian Journal of Agricultural Research* **32**, 99–108. doi:10.1071/AR9810099

Satter LD, Slyter LL (1974) Effect of ammonia concentration on rumen microbial protein production *in vitro. The British Journal of Nutrition* **32**, 199–208. doi:10.1079/BJN19740073

Thornton RF, Minson DJ (1973) The relationship between apparent retention time in the rumen, voluntary intake and apparent digestibility of legume and grass diets in sheep. *Australian Journal of Agricultural Research* **24**, 889–898. doi:10.1071/AR9730889

Weston RH (1996) Some aspects of constraint to forage consumption by ruminants. *Australian Journal of Agricultural Research* **47**, 175–197. doi:10.1071/AR9960175