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Effect of varying the proportion of molasses in the diet on intake, digestion and microbial protein production by steers

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Abstract. The present experiment was conducted to determine the efficiency of microbial protein production in the rumen and intake by cattle fed high-molasses diets. Intake and microbial crude protein (MCP) production were measured along with the concentration of rumen ammonia-nitrogen (N) and volatile fatty acids (VFA), pH and the rate of digestion of roughage in the rumen. Eight Brahman crossbred steers weighing 211 ± 19.3 (± s.d.) kg were used in a double 4 × 4 Latin square design. Steers were allocated to one of four total mixed rations: control (pangola hay only), 25M (25% molasses/urea mix + 75% hay), 50M (50% molasses/urea + 50% hay), and 75M (75% molasses/urea + 25% hay). The production and efficiency of production of MCP (EMCP) of the diet increased quadratically as the level of molasses in the diet increased. The EMCP from the molasses/urea mix was estimated as 166 g MCP/kg digestible organic matter (DOM), a relatively high value. Intake of dry matter (DM) and DOM increased quadratically, reaching a peak when molasses was ~50% (as fed) of the ration. Digestibility of DM increased quadratically and that of neutral detergent fibre decreased linearly with increasing level of molasses in the diet. Molasses inclusion in the diet had no effect on rumen pH, ammonia and VFA concentration in the rumen fluid, plasma urea-N, urine pH or ruminal fractional outflow rate of ytterbium-labelled particles and Cr-EDTA. It was concluded that a diet with a high level of molasses (>50%) and supplemented with adequate N had high EMCP, and that low MCP production was not a factor limiting intake or performance of cattle consuming high-molasses diets.

Additional keywords: efficiency, microbial protein production.

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Introduction

Molasses is often an inexpensive and readily available energy source for cattle production in tropical countries. In Cuba, high-molasses diets were successfully used for fattening beef cattle (Preston *et al*. 1967; Preston 1972). In Australia, molasses is used in drought feeding systems (Wythes and Ernst 1984) and has the potential to be used as a major energy source in diets for intensive finishing systems (McLennan *et al*. 1998; Hunter 2012). When molasses is included in the diet at low levels, i.e. <~20% of total diet dry matter (DM), the feeding value of the ration can improve (Van Niekerk and Voges 1976; Givens *et al*. 1992; Yan *et al*. 1996), whereas at higher levels, molasses is sometimes poorly utilised by ruminants (Lofgreen and Otagaki 1960).

Inclusion of true protein sources such as fish meal and cottonseed meal in molasses-based diets containing urea has significantly improved cattle performance (Preston *et al*. 1970; Preston 1972), suggesting that in their absence, high-molasses diets supply a low level of metabolisable protein to the animal. This further suggests a low efficiency of microbial crude protein (MCP) production (EMCP). If this is so, the improved performance of animals supplemented with true protein could be due to an increase in the supply of undegraded dietary protein to the animal (Preston 1972). The EMCP could also be increased in response to peptides, amino acids and branched-chain fatty acids formed during protein degradation (Hume 1970).

From first principles, diets high in molasses and rumen degradable nitrogen (RDN) should support high production of MCP due to the abundant supply of fermentable sugars and RDN in the rumen (CSIRO 2007). However, the literature on this topic is limited and shows highly variable results. Ramirez and Kowalczyk (1971) estimated the EMCP in calves fed a molasses/urea-based ration, which accounted for ~83% of the

total DM intake, to be 27.2 g microbial N/kg DM digested in the rumen (DMDR), similar to values determined by Lawson *et al*. (1986) for a grain-based concentrate diet (28.6–36.7 g microbial N/kg organic matter (OM) digested in the rumen). However, they estimated endogenous N flow to the duodenum using equations developed for use in sheep, not cattle, and thus overestimated EMCP. By comparison, Rowe *et al*. (1980), using bulls fed on similar high-molasses diets to those in the work of Ramirez and Kowalczyk (1971), reported much lower values of EMCP (14.8 g microbial N/kg DMDR), but even this was an overestimate as it was calculated on the assumption that all N entering the duodenum was of microbial origin. Nevertheless, Chamberlain *et al*. (1993) found the increase in EMCP on silage-based diets to be greater with the addition of sugars compared with starch, supporting the high potential of molasses as a substrate for microbial protein production.

Some of the variability in reported effects on EMCP with the inclusion of molasses in the diet could be related to the proportion of molasses used and/or the inclusion of other substrates in the diets. The present experiment was carried out to investigate the effect of molasses inclusion rates in diets for cattle on intake and MCP production in the rumen.

Materials and methods

Animals, experimental design and treatments

The experiment was carried out at the University of Queensland Mt. Cotton Research Farm (153°14′E, 27°53′S) between October 2002 and March 2003. Eight Brahman crossbred steers, ~14 months of age and weighing 211 ± 19.3 (± s.d.) kg were used. Four of the eight steers were fitted with a permanent rumen cannula (11 cm internal diameter; Bar Diamond Inc., Parma, ID, USA) 4 weeks before the start of the first experiment; the other four steers remained intact. The steers were treated with moxidectin (Cydectin; Fort Dodge Australia Pty Ltd, Sydney) to control internal and external parasites and were vaccinated against bovine ephemeral fever. During an adaptation period before the start of the experiment, steers were fed a molasses–hay combined diet during which time the proportion of molasses was increased from 0 to 50% (w/w, as fed) over 14 days. At the start of the experiment, steers were allocated by stratified randomisation based on liveweight to one of four diets in a double 4 × 4 Latin square design (one for fistulated and one for intact steers) with four runs of 28 days duration. In each run, the steers were fed in individual pens over a 21-day preliminary period and then transferred to metabolism cages for a 7-day collection period. The steers were weighed once weekly.

The experimental diets comprised varying proportions (w/w, as fed) of a molasses mix and chaffed pangola grass (*Digitaria eriantha*) hay: 0 : 100 (control), 25 : 75 (25M), 50 : 50 (50M) and 75 : 25 (75M). The molasses mix (141 g/kg DM CP equivalent) contained (w/w, as fed) 96% molasses, 3% urea and 1% monosodium phosphate and was prepared weekly by thoroughly mixing urea and anhydrous monosodium phosphate (Redox Chemicals Pty Ltd, Sydney), dissolved in hot water (1 kg/L), into the molasses using a pump. The molasses mix (hereafter called molasses) was re-mixed using this pump every day before feeding.

Experimental procedures

Diets were prepared daily by thoroughly hand-mixing the molasses and the hay into a homogeneous mixture. For each steer, the amount of total feed offered each day was set at 15% more than that consumed on the previous day. The steers were fed once daily at 0800 hours and had free access to fresh water at all times. Feed intake was determined daily. Separate samples of the molasses and hay offered during the collection period were taken daily, bulked and subsampled for DM determination and chemical analysis. Feed refusals were weighed daily, stored in a cold room (5−8°C), and bulked for each steer at the end of the collection period. These were thoroughly mixed and subsampled for determination of DM, chemical analysis and estimation of molasses intake. Subsamples of hay and refusals from the control and 25M treatments were dried, as collected, to constant weight in a forced-draft oven at 60°C, but drying of feed refusals by this method did not result in attainment of constant weight with the 50M and 75M treatments due to their high molasses content and formation of a barrier on the surface of the molasses. Consequently, an approximately equal weight of water was added to, and mixed with, a weighed subsample of these residue feeds, and a small weighed amount of oven-dried paper towel was added to take up the molasses/water solution. This total mixture (chaff, molasses and paper towel) was dried to constant weight (~7 days) as described above. A similar procedure was used for determining DM content of the molasses fed out.

There was some separation of molasses and hay in the mixed rations in feed troughs. To estimate molasses content of refusals and thereby calculate separate intakes of the hay and molasses components, the refusals were subsampled in triplicate and washed through tap water until the water was clear, and the remaining hay was dried to constant weight. The proportion of hay in the mixed feed refusals was calculated. To correct for the loss of dust and fine particles during the washing process, a subsample of the control diet was handled in the same way and the proportion of DM loss was used as a correction factor. To determine OM and neutral detergent fibre (NDF) contents in feed refusals, subsamples were weighed and the molasses was washed out as above. The remaining material was then dried, re-weighed, ground (1-mm screen) and analysed for OM and NDF content. This represented the total NDF in feed refusals, as molasses has no NDF content, whereas the OM content in feed refusals was calculated using the calculated proportions of molasses and hay in residue feed (see above) and their respective OM contents.

Faeces for each steer were collected daily and mixed thoroughly before a 5% aliquot was taken and frozen (−20°C), and separate duplicate subsamples were dried (60°C) to constant weight for DM determination. At the end of the collection period, the frozen samples were thawed, bulked for each steer and mixed, and a subsample was taken. The subsample was freeze-dried and ground (1-mm screen) before analysis for OM and NDF content. The digestibility of DM, OM and NDF was calculated. The daily urine output of individual steers was measured by collection into trays placed under the cages. The pH of the urine was kept <3.0 by collecting into ~300 mL of a 25% sulfuric acid solution. A 5% aliquot of the total daily collection was taken and added to a bulked sample for each steer and kept frozen over the collection period. At the end of each run, the

bulked samples were thawed and mixed, and one 50-mL subsample was frozen undiluted while another 5-mL subsample was diluted with 45 mL of 0.1 M ammonium phosphate stock buffer solution and then frozen. Both samples were kept for later analysis for purine derivative (PD) concentrations, but only the acidified initial sample was used in analysis. Long-term storage of the buffered solution, but not the acidified sample, was shown to affect the concentration of PD. The acidified sample was thawed, buffered and analysed immediately by the PD method (Balcells *et al*. 1992; Chen and Gomes 1995). Fresh non-acidified urine samples were collected from all steers at two times (0900–1100 hours and 1500–1700 hours) over two consecutive days of the collection period, and the pH was determined immediately.

Samples of rumen fluid (~100 mL) were taken from the four fistulated steers every 4 h over 24 h on day 5 of the collection period, using a sampling probe inserted through the cannula. Rumen fluid was drawn from frontal, caudal and ventral sites of the rumen and mixed. The pH of rumen fluid was measured immediately after sampling. One 4-mL subsample of rumen fluid from each steer was mixed with 4 mL of 0.2 N HCl, and another 4-mL subsample was mixed with 1 mL metaphosphoric acid internal standard buffer solution. Both samples were stored at −20°C for later determination of ammonia-nitrogen (NH_3-N) and volatile fatty acid (VFA) concentrations, respectively. Samples collected for determination of VFA concentration were later thawed and bulked across sampling times for each steer before analysis. Changes in rumen pH and concentrations of NH_3-N were analysed between 0 and 20 h, thereby avoiding bias resulting from including the 0 or 24 h value twice.

The fractional outflow rate (FOR) of fluid from the rumen was determined using chromium-EDTA (Cr-EDTA) as a marker. The Cr-EDTA solution was prepared by the method of Downes and McDonald (1964). Passage rate of the solid phase of the digesta was determined using ytterbium (Yb) as a marker, together with collections from both the rumen and faeces. The Yb marker was prepared by dissolving ytterbium chloride hexahydrate in deionised water (35.8 g made up to 1 L), spraying this solution evenly over pangola hay ground though a 1-mm screen, and mixing thoroughly (de Vega and Poppi 1997). Further deionised water was added to make a slurry, and this was left for 12 h and then squeezed through a sieve of pore size 150 µm to separate water and the finer particles from the mixture. The remaining material was dried in an oven at 60°C for 24 h, mixed again and then made up into ~12-g parcels using a paper towel. Blank rumen fluid, digesta and faecal samples were taken before injection of markers as background values for analysis of Cr and Yb concentrations. At 08:00, Cr-EDTA solution was injected into four sites in the rumen of the fistulated steers, thereby delivering a dose of ~2 g Cr. At the same time, six parcels of Yb-labelled particles, wrapped in paper towel and containing in total 0.7–0.9 g Yb, were inserted into the rumen of each fistulated steer at different sites and torn by hand to expose the particles. Rumen fluid samples were taken at 4, 8, 12, 16, 20, 24, 28, 32, 36 and 48 h after dosing. Duplicate 10-mL subsamples were taken at each sampling time and frozen before analysis for Cr concentration. Rumen digesta samples were taken simultaneously with rumen fluid from the frontal, caudal and ventral sites in the rumen. These samples were initially frozen, then thawed, bulked across sampling sites for each steer, oven-dried (60°C) to determine DM content, ground (1-mm screen) and then analysed for Yb concentration. Faecal samples were taken via the rectum every 12 h over a period 24–96 h after marker dosing. These samples were initially frozen, then thawed and oven-dried (60°C), ground (1 mm screen) and analysed for Yb concentration.

Blood samples were taken from the tail vein of all steers at 4 h after feeding on the last day of each collection period. The samples were centrifuged at 700*g* for 15 min and the plasma was removed and stored at −20°C before analysis for plasma urea-N concentration.

The rate of digestion of pangola hay was determined *in situ* between days 16 and 20 of the preliminary period. Approximately 4 g of ground (3-mm screen) pangola hay was inserted in nylon bags, 24 by 10 cm with a pore size of 45 µm, and incubated, in triplicate, in the rumens of the four fistulated steers for 0, 4, 8, 12, 24, 48, 72 and 96 h. Upon removal from the rumen, bags were lightly washed to remove digesta from the outside and immediately frozen. At the end of the study, bags were defrosted in cool water and uniformly washed and squeezed under running water before undergoing a final fixed-duration wash and spin-dry in a washing machine. The bags were oven-dried (60°C for 48 h), cooled in a desiccator and weighed to determine the proportional DM loss. The potential degradability and rate of degradability of DM were determined according to the equation of Ørskov and McDonald (1979), $p = a + b\ (1 - e^{-ct})$, where p is the proportion of total DM degraded at time t, a is the intercept at time 0 h representing the immediately soluble component, b is the insoluble but potentially degradable fraction, and c is the fractional rate constant of degradation of the b fraction.

Laboratory analysis

Ash content was determined by combusting ~1 g of oven-dried, ground (1-mm screen) sample in a muffle furnace at 550°C for 4.5 h. The ash-free NDF and ash-free acid detergent fibre (ADF) content of hay, and ash-free NDF content of feed refusals and faeces (freeze-dried) were determined by the method of Van Soest and Wine (1967) using a fibre extraction unit (ANKOM 220; ANKOM Technology, Macedon, NY, USA) followed by combustion to determine ash content, as described above. The N concentration in the hay and the molasses mix was determined using an automatic total N analyser (FP-428; LECO Australia, Castle Hill, NSW). Using methods described in Panjaitan *et al*. (2010*a*), the concentration of NH_3-N in the rumen fluid was determined by a distillation method (with sodium tetraborate to elevate pH) and the concentration of VFA and branched-chain fatty acids (BCFA) with a gas chromatograph. The plasma urea-N concentration was measured using an enzymatic kit method (Thermo Electron Corporation, Noble Park, Vic.). The concentration of PD in urine was determined using high-performance liquid chromatography according to the method of Balcells *et al*. (1992), and microbial protein production was calculated by the method of Bowen *et al*. (2006) using their value for endogenous urinary PD excretion for *Bos indicus* cattle.

Two determinations of EMCP were made; one as MCP production relative to digestible OM intake (DOMI) from the

total diet, and the other as MCP production relative to DOMI from the molasses component alone. For the latter, a regression equation was first established for steers on the control treatment (nil molasses) across runs relating OM digestibility (OMD) to NDF digestibility (NDFD). This equation was then applied to the other treatments which included molasses, to estimate OMD of the hay component of the diet from known NDFD of the total diet, which was attributable solely to the hay, as molasses has no NDF, and assuming that the above, established regression was not affected by presence of the molasses. From this OMD value and hay intake estimated as described above, DOMI from the hay was calculated and by difference from total DOMI, the DOMI from the molasses component was derived. The MCP production from the molasses component was calculated by further assuming that the EMCP attributable to the hay component was constant across all diets and equal to that of the control treatment.

Rumen fluid was centrifuged at 1790*g* for 10 min and the concentration of Cr in the supernatant was determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AES) (SpectroFlame P; SPECTRO Analytical Instruments GmbH, Kleve, Germany). These values were corrected using a standard curve prepared using known concentrations of Cr and a blank from rumen fluid collected before the Cr-EDTA injection. The concentration of Yb in oven-dried faeces and digesta samples was also measured with the ICP-AES instrument following an acid-digest using a mixture of 5 : 1 nitric acid : perchloric acid and dilution to known volume with deionised water (de Vega and Poppi 1997). Standard curves for Yb were prepared using the same procedure following the incremental addition of Yb in measured amounts to samples of rumen digesta and faeces collected pre-injection. Since a single-dose approach was used, concentrations of Yb and Cr in the rumen over time followed an exponential decay pattern. The natural logarithm (ln) of concentrations was plotted against time, and the slope of the linear equation was used to estimate the FOR of liquid and solid phase markers, Cr and Yb, respectively.

Statistical analyses

The statistical significance of the effects of level of molasses in the diet on each of the variables was tested by analysis of variance with terms for animal, run and level of molasses. For the variables that were recorded on both intact and fistulated steers, terms for steer type and its interaction with level of molasses were also included. The effects of level of molasses were partitioned into linear and quadratic components, and the highest statistically significant ($P < 0.05$) component was used as the degree of polynomial for illustration of the response. Pairwise comparisons between means used the protected l.s.d. procedure. Due to high variability in the rumen NH_3-N concentrations, especially at 4 h post-feeding, the data were ln-transformed before analysis. The statistical package GENSTAT (GENSTAT 2006) was used for all analyses.

Results

The DM contents of molasses, molasses mix and pangola hay were 755, 763 and 888 g/kg, respectively. The chemical composition of the molasses mix offered (g/kg DM) was 832 OM and 22.6 N, and that of pangola grass hay 935 OM, 13.9 N, 711 NDF and 416 ADF. Table 1 shows the composition of the various diets in terms of the contributions from molasses mix and pangola hay and the overall diet chemical analysis. The CP (N × 6.25) content increased gradually, and NDF decreased sharply, as the proportion of molasses mix increased.

Production of MCP in the rumen and the estimated EMCP, based on total DOMI or on DOMI from the molasses component only, are presented in Table 2. The estimated supply of MCP to the animals, expressed either as g/day or as a function of liveweight, and the EMCP (total diet) increased quadratically with increasing proportion of molasses in the diet, and peak MCP production occurred when molasses comprised ~50% of the ration (as fed). There were no significant differences between diets in the estimates of EMCP attributable to the molasses component of the diet.

The intake of OM and digestible OM (DOM) increased quadratically with increasing proportion of molasses in the diet, reaching a peak when the molasses mix constituted ~50% (as fed) of the diet (Table 3). Conversely, there was a linear decline in the intake of NDF with increasing molasses contribution in the diet DM (Table 3). There was a quadratic increase in the digestibility of DM and OM, but a linear decrease in NDFD as the level of inclusion of molasses in the diet increased (Table 3).

When steers were fed rations including molasses, the pH in rumen fluid declined sharply in the first 4 h after feeding in the morning, although at all times exceeding 6.2, and then increased gradually over the remainder of the day to peak at ~7.0–7.2 just before the next feeding (Fig. 1). There was a more gradual reduction in pH for the control group but pH still declined to ~6.4 at 12 h. There were no treatment effects on rumen pH overall ($P > 0.05$; Table 4) and no interaction between treatment and time after feeding ($P > 0.05$).

The average concentrations of NH_3-N in rumen fluid across sampling times were not different between treatments (Table 4) but there was a significant treatment × sampling time

Table 1. Composition of the diets consumed as molasses mix and pangola grass hay (g/kg total dry matter (DM) intake) and their chemical analyses (g/kg DM, unless otherwise indicated) and estimated energy density (M/D)

Treatments: control, 25M, 50M and 75M included 0, 25, 50 and 75% molasses mix (w/w, as fed) combined with pangola grass hay. OM, Organic matter; NDF, ash-free neutral detergent fibre; CP, crude protein (nitrogen × 6.25)

	Control	25M	50M	75M
Diet composition				
Molasses	0	223	462	720
Pangola hay	100	777	538	280
Chemical composition of diets				
DM (g/kg)	888	857	826	795
OM	935	912	887	861
NDF	711	553	382	199
CP	87	99	112	126
M/D (MJ/kg DM)[A]	8.4	8.6	9.1	10.1

[A]Energy density of the diets estimated from equation: M/D (MJ/kg DM) = 0.0157 Digestible OM in DM (DOMD; g/kg DM) (AFRC 1993) with the DOMD derived from the current experiment.

Table 2. Effect of varying the proportions of molasses and pangola hay in the diet of steers on their microbial crude protein (MCP) production and production efficiency (EMCP) expressed relative to total digestible organic matter (DOM) intake or to DOM from the molasses component of the diet only

Treatments: control, 25M, 50M and 75M included 0, 25, 50 and 75% molasses (w/w, as fed) combined with pangola grass hay. Within rows, means followed by the same letter are not significantly different at $P = 0.05$; l.s.d., protected least significant difference ($P = 0.05$); *P*-values for the linear and quadratic coefficients in the regression equations; $^*P < 0.05$; $^{**}P < 0.01$; n.s., not significant ($P > 0.05$)

Measurement	Control	25M	50M	75M	l.s.d.	Linear	Quadratic
MCP production							
(g/day)	210a	332b	458c	437c	51.7	**	**
(g/kg LW.day)	0.88a	1.41b	1.95c	1.93c	0.220	**	**
EMCP							
(g MCP/kg DOM total)	82.7a	111.2b	131.8c	137.5c	15.41	**	*
(g/MCP.kg DOM molasses)	–	172.8	173.4	151.5	40.53	n.s.	n.s.

Table 3. Effect of varying the proportions of molasses and pangola hay in the diet of steers on intake and digestibility

Treatments: control, 25M, 50M and 75M included 0, 25, 50 and 75% molasses (w/w, as fed) combined with pangola grass hay. DM, Dry matter; OM, organic matter; DOM, digestible OM; NDF, neutral detergent fibre. Within rows, means followed by the same letter are not significantly different at $P = 0.05$; l.s.d., protected least significant difference ($P = 0.05$); *P*-values for the linear and quadratic coefficients in the regression equations; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant ($P > 0.05$)

Measurement	Control	25M	50M	75M	l.s.d.	Linear	Quadratic
Intake (g/kg LW.day)							
Molasses DM	0a	5.8b	11.7c	15.7d	1.58	**	n.s.
Total DM	20.5a	23.5ab	26.1b	22.3a	3.16	n.s.	**
OM	19.1a	21.2ab	23.1b	19.1a	2.88	n.s.	**
DOM	10.9a	12.7b	14.9c	14.2bc	1.81	**	*
NDF	14.6a	12.6b	10.2c	4.7d	1.67	***	**
Digestibility (g/100 g)							
DM	58.9a	62.7b	68.4c	77.0d	1.71	**	**
OM	57.4a	60.6b	65.5c	74.7d	1.38	**	**
NDF	62.8a	56.5b	53.0c	46.5d	2.31	**	n.s.

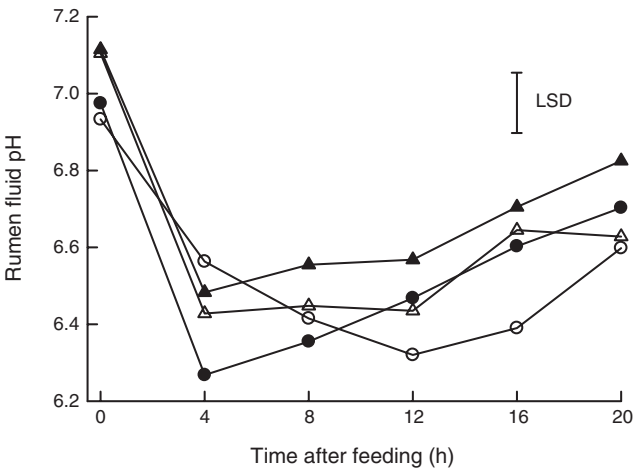

Fig. 1. Changes in pH in the rumen fluid with time after feeding (0800 hours) for steers receiving diets comprising molasses and pangola grass hay in proportions (w/w, as fed) of 0 : 100 (control; ○), 25 : 75 (25M; ●), 50 : 50 (50M; △) and 75 : 25 (75M; ▲). Points shown on the graph are treatment means for steers across runs. l.s.d., protected least significant difference ($P = 0.05$).

interaction ($P < 0.01$). Owing to the high within-treatment between-animal variability, a ln-transformation of data was carried out and the trends are illustrated in Fig. 2. The main feature of the diurnal changes was a rapid increase in NH_3-N concentration soon after feeding, so that there were no differences between groups receiving molasses at 4 h ($P > 0.05$), but with the exception of the 50M group, these concentrations were greater than for the control group at this time. Thereafter, concentrations tended to decline, and from 4 h, differences between these groups and the control were generally non-significant. The exception was for the 25M group, which had a lower concentration than other groups from 12 h onwards, significantly ($P < 0.05$) so at 16 and 20 h except in relation to the 50M group at 20 h. The total concentration of VFAs in rumen fluid averaged 45.4 mmol/L across all groups and was not affected by diet (Table 4). Similarly, although there was a trend for the molar proportion of acetate to be lower and of butyrate higher ($P = 0.08$) for the 75M steers compared with other groups, these differences were not significant.

Plasma urea-N was similar for groups with molasses in the diet ($P > 0.05$) and was higher overall than for the control ($P < 0.05$), although the difference between the control and 50M treatments

Table 4. Effect of varying the proportions of molasses and pangola hay in the diet on the pH and the concentrations of ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFA), and the molar proportions of individual VFA (% of total VFA), in the rumen fluid and on the output and pH of urine and on the concentration of urea-N in plasma (PUN) of steers

Treatments: control, 25M, 50M and 75M included 0, 25, 50 and 75% molasses (w/w, as fed) combined with pangola grass hay. Within rows, means followed by the same letter are not significantly different at $P = 0.05$; l.s.d., protected least significant difference ($P = 0.05$); *P*-values for the linear and quadratic coefficients in the regression equations; *$P < 0.05$; n.s., not significant ($P > 0.05$)

	Control	25M	50M	75M	l.s.d.	Linear	Quadratic
Rumen pH	6.54	6.56	6.62	6.71	0.152	n.s.	n.s.
NH_3-N concentration (mg/L)	76.6	83.8	99.2	107.8	41.7	n.s.	n.s.
Total VFA concentration (mmol/L)	44.9	46.4	42.1	48.0	10.57	n.s.	n.s.
VFA molar proportion (%)							
Acetate	64.2	68.5	64.6	56.3	12.46	n.s.	n.s.
Propionate	23.9	18.5	24.1	26.8	11.71	n.s.	n.s.
Butyrate	10.3	11.2	9.7	15.8	5.63	n.s.	n.s.
Valerate	0.81	0.74	0.66	0.82	0.379	n.s.	n.s.
BCFA[A]	0.81	1.12	1.01	0.30	1.031	n.s.	n.s.
Urinary output (L/day)	9.5a	15.2b	21.5c	27.6d	4.12	*	n.s.
Urine pH	7.63	7.71	7.46	7.21	0.622	n.s.	n.s.
PUN concentration (mmol/L)	2.56a	3.79b	2.99ab	3.85b	0.945	*	n.s.

[A]Branched-chain fatty acids (isobutyrate + isovalerate).

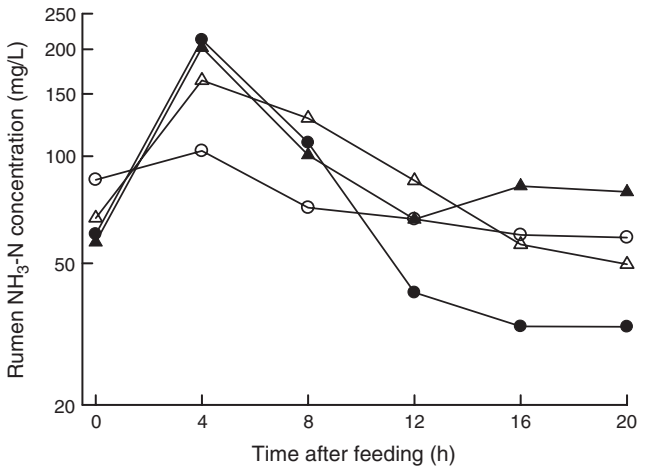

Fig. 2. Changes in the concentration of ammonia-nitrogen (NH_3-N) in the rumen fluid with time after feeding (0800 hours) for steers receiving diets comprising molasses and pangola grass hay in proportions (w/w, as fed) of 0 : 100 (control; ○), 25 : 75 (25M; ●), 50 : 50 (50M; △) and 75 : 25 (75M; ▲). Points shown on the graph are treatment means for steers across runs. Values for NH_3-N concentrations are treatment means (geometric means) back-transformed after the analysis of variance of ln-transformed data.

was not significant. Daily urine output increased linearly with increasing levels of molasses in the diet, such that for the 75M treatment, there was a 3-fold increase in urine output compared with the control (Table 4). The mean pH value of urine was not affected by treatment ($P > 0.05$).

There was a trend for the FOR of the particle phase to increase with increasing molasses inclusion in the diet when determined using the Yb concentration in digesta samples ($P = 0.055$) but not in the faeces ($P > 0.05$). There was no effect of diet on FOR of Cr-EDTA (Table 5).

There was no effect of level of inclusion of molasses in the diet on the rate of digestion of hay DM *in situ*, although the 75M

Table 5. Effect of varying the proportions of molasses and pangola hay in the diet on the fractional outflow rates (FOR, %/h) of ytterbium (Yb)-labelled pangola hay particles released in the rumen and subsequently collected in rumen digesta (RD) or faecal samples, and of chromium (Cr)-EDTA

Treatments: control, 25M, 50M and 75M included 0, 25, 50 and 75% molasses (w/w, as fed) combined with pangola grass hay. l.s.d., protected least significant difference ($P = 0.05$); *P*-values for the linear and quadratic coefficients in the regression equations; n.s, not significant ($P > 0.05$)

	Control	25M	50M	75M	l.s.d.	Linear	Quadratic
FOR of Yb (RD)	4.7	4.9	5.4	6.0	1.38	0.055	n.s.
FOR of Yb (faecal)	3.4	4.7	4.9	4.7	1.30	n.s.	n.s.
FOR of Cr-EDTA	8.8	10.0	9.0	9.2	2.58	n.s.	n.s.

treatment tended to be associated with a longer lag time than all other diets (3.7 *v.* 2.3–2.8 h; $P = 0.075$). The average value for 'a', the immediately soluble component, was 9.9% (s.e.m. 0.59); for 'b', the insoluble but potentially degradable fraction, 62.2% (s.e.m. 0.60); and for 'c', the fractional rate of digestion, 0.046 h^{-1} (s.e.m. 0.002).

Discussion

Microbial protein production

Molasses has been widely used as a relatively inexpensive energy source for cattle in tropical countries. Being a source of readily fermentable sugars (Wythes *et al*. (1978), it should support high production of microbial protein and a high efficiency of MCP production in the rumen of cattle provided there is an adequate supply of rumen degradable N. Nevertheless, it has been commonly observed that cattle fed high-molasses diets respond in intake and growth rate to the inclusion of an undegraded dietary protein source (Preston *et al*. 1970; Preston 1972), suggesting low production of MCP in the rumen. There are, however, few and contradictory reports of the efficiency of

conversion of fermentable energy and degradable protein to MCP for cattle receiving diets with high molasses content. This provided the research basis for the current experiment.

Our results show that for diets with moderate to high rates of inclusion of molasses, the efficiency of MCP production was within the expected range outlined in the feeding standards. When molasses comprised ≥50% of the feed on offer, the EMCP ranged from 132 to 138 g MCP/kg DOM, consistent with the range 130–170 g/kg DOM proposed by AFRC (1993) and CSIRO (2007). Diets containing less molasses (0–25%) were associated with EMCP values below this range, as has been shown previously with tropical forages (Panjaitan *et al*. 2010*b*).

The estimate of EMCP attributable to the molasses component of the diets, which was calculated on the assumption that the EMCP from the hay component remained constant for all diets and equivalent to that for the pangola grass hay alone (control, 82.7 g MCP/kg DOM), averaged 166 g MCP/kg DOM across diets. This is in the upper range of values reported from the feeding standards. It should be noted that in our experiment, EMCP was expressed as MCP produced relative to the OM digested in the total gastrointestinal tract. A more precise value would result from expressing it relative to the OM truly digested in the rumen, or fermentable metabolisable energy (ME), but total tract DOM was used here as it could be actually measured. If values were expressed relative to OM fermented only in the rumen, the EMCP attributable to the hay component would be slightly higher than the 82.7 g MCP/kg DOM reported in Table 2, accounting for post-ruminal digestion of the forage, meaning that values determined for the molasses component would be slightly lower than those calculated above on the basis that all soluble carbohydrate of molasses is reportedly digested in the reticulo-rumen (Geerken and Sutherland 1969; Ramirez and Kowalczyk 1971).

Of the few other reports on EMCP from *in vivo* studies using molasses-based diets, Ramirez and Kowalczyk (1971) provided values of similar order to our results. When young calves were fed molasses and urea (3% w/w of molasses) and a restricted amount of rice straw (2.7 g/kg liveweight (LW).day) the estimated EMCP was 156 g MCP/kg ruminally fermentable carbohydrate. Our values were calculated from the method based on urinary excretion of PD (Chen and Gomes 1995) but we used the lower value for endogenous PD excretion of 0.190 mmol/kg $LW^{0.75}$.day, based on the work of Bowen *et al*. (2006), who determined that endogenous production in *Bos indicus* steers was much lower than the value of 0.385 mmol/kg $LW^{0.75}$.day suggested by Chen and Gomes (1995) for cattle in general. In another study, Rowe *et al*. (1980) fed bulls a molasses/urea-based diet supplemented with forage and meal from cassava, in which the molasses/urea accounted for ~70% of the DM intake. They estimated EMCP to be 56 g MCP/kg DM apparently fermented in the rumen. This value, although very low, may have still been an overestimation as it was based on the assumption that all N entering the duodenum was of microbial origin, thereby ignoring contributions from NH_3, endogenous and dietary N sources to the flux of N in the duodenum. Although the EMCP attributable to the molasses alone could not be calculated from their results, it appears to be much lower for their feeding regime than that of the present experiment and well outside the expected values from the feeding standards.

Apart from the energy supply, MCP production is closely aligned with supply of RDP to the rumen microbes. Assuming CP degradability in the rumen of 87% for pangola hay (Bowen 2003) and 100% for the urea within the molasses (NRC 1996), the RDP supply for the control, 25M, 50M and 75M diets was estimated to be 140, 163, 181 and 185 g/kg DOM, respectively, initially suggesting adequate RDN for optimal microbial growth relative to EMCP values in the feeding standards (130–170 g MCP/kg DOM). However, based on measured EMCP values, the capture of RDP by rumen microbes averaged only 59, 68, 72 and 74% for the four diets, respectively. The relatively small increase in the rate of incorporation of RDP with diets including molasses in particular suggests that urea concentration, relative to fermentable energy, was higher than optimum or that fermentable ME of the diets was low. However, if it is assumed that the RDP capture from the hay component was constant (59%), the corresponding RDP capture from the molasses/urea mix averaged 80%, agreeing with the value suggested by AFRC (1993) for conversion of non-protein N sources such as urea to MCP.

Intake and digestibility

The intake of DM and DOM did not increase linearly with increasing molasses content in the diet, instead increasing quadratically such that peak values for DMI and DOMI of 26.1 and 14.9 g/kg LW.day occurred when molasses contributed 50% of the fresh weight of the diet. McLennan (1992) recorded a similar DMI when molasses comprised 64% of the total diet for Brahman crossbred weaner steers, but in another study, McLennan *et al*. (1998) recorded a much higher intake of 32.8 g/kg LW.day when molasses comprised 63% of the diet of Hereford steers. Both diets included whole cottonseed as a protein and lipid source, and in both experiments, the molasses mix was fed separately from the other feed components. Hunter (2012), who fed a total mixed ration, found a maximum DMI of 27.2 g/kg LW.day when molasses was included at 45% dietary DM. The recorded DOMI for 50M and 75M rations equates to estimated ME intakes of 54.7 and 51.5 MJ/day (see Tables 1, 3), which, from AFRC (1993), should result in a liveweight gain of ~0.75 kg/day. These values lead to consideration of what might be the factors limiting intake of the steers on these high-molasses diets.

The intake levels we recorded appear lower than physically possible when considered on the basis of NDF content and in relation to other diet types. For example, in the 50M ration the NDF content of the mixed diet was 382 g/kg DM, which can be compared with the values for sheep of 194 g/kg for white clover and 344 g/kg for ryegrass (Cruickshank *et al*. 1992) and a recommendation of at least 250 g/kg for total mixed rations for high-producing dairy cows (Mertens 1997). At 75% molasses inclusion, the NDF content was 199 g/kg DM. The response of intake, both total and hay intake, to increasing levels of molasses in the current experiment appears remarkably similar to that observed in lambs fed a total mixed ration with pangola hay and sucrose added in varying proportions from 0 to 60% inclusion, which was quadratic in nature (de Vega and Poppi 2012). Yet in both experiments, intake did not approach that

achieved when whole cottonseed, which has both undegraded protein and a significant level of lipid, was used as a supplement with high-molasses diets (McLennan *et al*. 1998; Hunter 2012). On the other hand, metabolic control of intake, whereby ME intake is close to the genetic potential for growth of these steers, also seems unlikely given that the above estimated ME intakes are much lower than this potential (Weston 1996). Other factors seem more likely to restrict intake on this diet type.

A range of other possible metabolic and physiological factors might restrict intake by cattle of high-molasses diets. Supply of MCP and the MCP/DOM ratio (i.e. the P/E ratio) are within normal limits for a variety of forage types, so this nutrient balance is unlikely to affect intake. Initially we hypothesised that this was a possible regulator of intake of molasses-based diets if EMCP was found to be low, but this does not appear to account for differences in intake. Rumen pH was always >6.2 and thus was unlikely to affect intake (Forbes 1986). Rumen stasis would also appear not to be an issue as the FOR of both Yb-labelled particles and Cr-EDTA were within an expected range and not depressed by level of molasses (Table 5; Beever *et al*. 1980–81; de Vega and Poppi 1997). Molasses has a high concentration of various minerals (K, Ca, Mg, Cl, S) and the high osmolarity associated with this might affect intake. Ternouth (1965) indicated that an increase in osmolarity of rumen liquor resulted in a reduction in voluntary feed intake and an increase in water consumption. In the present experiment, water intake was not measured but the volume of urine output increased linearly with increasing levels of molasses in the diet (Table 4), which suggests an associated higher water intake under high levels of molasses intake. In addition, Benavides and Rodriguez (1971) indicated that cattle fed molasses diets had a much higher water intake than cattle fed on roughage and/or concentrate diets. The FOR of Cr-EDTA in the rumen, however, was not affected by the molasses level as one would expect if osmolarity increased markedly. Beever *et al*. (1980–81) suggested that an increase in osmolarity of rumen liquor was associated with an increase in turnover rate of the rumen fluid. The minerals in molasses may also affect the acid–base balance within the animal in terms of the value for the cation–anion difference. However, urine pH (Table 4) was not affected by molasses level and the value did not indicate a change in the acid–base balance within the body of the animal.

The presence of a rumen mat and NDF characteristics were suggested by de Vega and Poppi (2012) as a limit to intake with high sucrose diets, and this may have contributed to intake regulation here. However, the values for substitution of NDF intake of the forage with increasing molasses inclusion in our study appear much greater than can be explained by a mat mechanism. In addition, faecal output of NDF declined markedly with level of molasses inclusion, indicating that the passage of NDF from the rumen was not restricted to a constant passage rate as expected if a mat mechanism prevailed. The reason for the lower than expected level of intake with high-molasses diets remains uncertain.

Preston (1982) indicated that low intake of high-molasses diets was the main limitation to their widespread use, and that use of undegradable true protein increased molasses intake. In support, McLennan *et al*. (1998) showed that the inclusion of cottonseed meal and whole cottonseed, incorporating rumen and undegraded protein as well as lipid, in a molasses-based diet increased LW gain to 1.2 kg/day with an intake of 32.8 g/kg LW. day. Similarly, Hunter (2012) reported LW gains of up to 1.5 kg/day when molasses comprised 60% of the diet and was supplemented with urea, cottonseed meal and whole cottonseed; however, both intake and LW gain declined when the molasses inclusion rate increased to 72.5%. Protein *per se* does not appear to be the major limitation given the MCP production values in this experiment, yet some unknown feature of the other diets appears to enhance intake. This experiment indicates that intake is still the major limitation to the successful use of high-molasses diets. Several factors, in particular MCP supply, have been eliminated as contributing factors by the results of the present experiment. Nevertheless some experiments have achieved high intakes and LW gain (McLennan *et al*. 1998; Hunter 2012) with inclusion of a supplement with high undegraded protein and lipid.

Digestibility of OM linearly increased with molasses concentration in the diet due to the higher digestibility of molasses than pangola hay. The digestibility coefficient of NDF, on the other hand, decreased linearly as molasses inclusion in the diets increased. This is in agreement with findings previously reported with molasses or sucrose supplements to hay diets, although at low levels up to ~15% inclusion there was little to no effect (de Vega and Poppi 2012). Thus, at low levels of inclusion in the diet, sugar and/or molasses have little effect on digestibility of NDF, whereas at high levels they depressed fibre digestion considerably.

There was an indirect relationship between digestibility of NDF and FOR of Yb-labelled particles, suggesting that a large part of the decline in NDFD was due to an increase in passage rate. The equation was: NDFD = 117 – 11.8(FOR of Yb) (R^2 = 0.95; P < 0.05). The activity of cellulolytic bacteria appears inhibited at rumen pH <6 (Mould and Ørskov 1983). However, in our experiment, rumen pH was maintained >6 at all times. Huhtanen and Khalili (1992) suggested that sucrose would depress carboxymethylcellulase activity, but there was no evidence of a change in rate of digestion of DM in the hay fraction with increasing levels of molasses in the current experiment.

Rumen fermentation

The change of rumen pH over 24 h was similar to the patterns observed by McLennan (1992) in cattle fed molasses-based diets. Carbohydrates in high-concentrate (grain) diets usually lower rumen pH to <6 shortly after feeding. However, high-molasses diets do not usually cause as large a decrease in pH as high-grain diets (Peron 1971). The rumen pH observed in the present experiment was always >6, in agreement with the above studies.

The variation in NH_3-N concentration over 24 h can be closely linked with the eating pattern of the steers. The highest concentration of NH_3-N occurred at 4 h after feeding, which reflected the amount of urea ingested with molasses from the diets soon after presentation. The NH_3-N concentration in the rumen of the steers fed molasses diets was always >50 mg NH_3-N/L, which is generally regarded as the minimum level of NH_3-N required for effective microbial growth (Satter and Slyter 1974). Plasma urea-N concentrations, measured 4 h after

feeding, closely reflected total N intake and the rumen NH_3-N concentration 4 h after feeding.

The lack of treatment effect on total concentrations and molar proportions of VFA was not expected. The overall VFA concentrations seem low considering the moderate total DM intakes (20–26 g/kg LW.day), and we cannot explain these low values. Previous studies have shown that high levels of molasses in the diet are associated with an increase in the molar proportion of butyric acid and a decrease in propionic and acetic acids (Marty and Preston 1970; Marty and Henderyckx 1973; McLennan 1992). This pattern was not observed in our study. However, in the aforementioned studies molasses was offered separately from other components. In this situation, when hay is fed at restricted levels, the hay component is often rapidly consumed followed by smaller meals of molasses throughout the day (McLennan *et al*. 1998). It is possible that in those studies there were some periods when molasses was almost the only substrate for microbes to use, and butyrate-producing bacteria might be more competitive (prolific and/or numerous) than propionate-producing bacteria. This was not the case in the present experiment, as the cattle were fed total mixed rations. Komkris *et al*. (1965) also indicated that the feeding method (molasses and hay fed separately or as a total mixed ration) could result in different fermentation patterns. McLennan and Leng (2003) also showed that the rate of intake of molasses, and thereby the method of feeding, could have a pronounced effect on the pattern of VFA in the rumen of cattle.

It was concluded that MCP production from molasses was at the higher end of the range of expected values from the feeding standards. Low intake is still the major limitation to the successful use of molasses in production systems for cattle but some dietary combinations are able to achieve high intakes by unknown mechanisms.

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