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Responses to various protein and energy supplements by steers fed low-quality tropical hay. 1. Comparison of response surfaces for young steers

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Abstract. Response curves were established for different supplements, offered at intakes ranging from 0 to 20 g/kg liveweight (W).day to young *Bos indicus* crossbred steers fed low-quality Rhodes grass (*Chloris gayana*) hay *ad libitum* in two pen experiments. Supplements included protein meals of varying rumen-degradability (cottonseed meal (CSM) or fishmeal), as well as 'energy sources' comprising grains of high and low ruminal starch degradability (barley and sorghum) and a highly fermentable sugar source (molasses), with all diets adjusted for rumen-degradable nitrogen and mineral content. Unsupplemented steers gained 0.08 and 0.15 kg/day, in Experiments 1 and 2, respectively. Growth of steers increased linearly with intake of 'energy source' supplements in increasing order of molasses, sorghum and barley (all differences $P < 0.05$). Steer growth rate also increased linearly with fishmeal, albeit over a narrow intake range (0–4.1 g/kg W.day), whereas the response with CSM was asymptotic, showing a steep response at low intake before levelling at ~1.2 kg/day. All supplement types were associated with a linear reduction in hay intake by the steers (energy substitution) where the reduction was greater ($P < 0.05$) for barley and molasses (not different) than for sorghum ($P < 0.05$), and for fishmeal compared with CSM ($P < 0.05$). In concurrent metabolism studies with the same rations, organic matter digestibility of the total ration (561–578 g/kg DM, unsupplemented) was increased linearly by barley and molasses (both $P < 0.05$) but was unaffected by CSM and sorghum supplements. The efficiency of microbial protein synthesis in steers increased linearly, from 91 g microbial crude protein/kg digestible organic matter (unsupplemented), in both molasses and CSM-supplemented steers, with the trend for a higher response to molasses ($P = 0.05$), and appeared most closely related to digestible organic matter intake. The response curves from these studies provide the practical framework upon which to formulate rations for cattle grazing low-quality forages.

Additional keywords: energy retention, metabolisable energy intake, plasma urea, response surface, rumen ammonia, substitution effects.

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Introduction

The growth of cattle grazing unimproved tropical native pastures in northern Australia becomes severely constrained during the dry season (winter/spring months) by the low quality of the available forage. Deficiencies of protein and digestible energy in particular, coupled with low mineral content, constrain whole-of-life growth rates of cattle resulting in delayed age of cattle at slaughter, reduced meat quality (Harper 1999) and low prospects for meeting the high weight-for-age targets of premium beef markets (Bortolussi *et al*. 2005). The provision of nutritional supplements to grazing cattle provides one option to overcome these limitations.

Although the major deficiencies of protein and energy are well recognised, commercial decisions on the choice of supplement for cattle will be based on the growth response to correcting these limiting nutrients, the relative cost of providing them in commercial supplements and the practicalities, including palatability, of delivering them under extensive grazing conditions. Various supplement choices are available and for convenience these are often colloquially termed protein or energy supplements, based on their main constituency, but this classification masks other important contributions they make to the nutrition of the animal. For instance, protein meals provide energy substrates as lipid and fibre and through

gluconeogenesis from amino acids whereas energy sources, although rich in fermentable starch or sugars, also contain protein and provide further amino acids to the animal by stimulating rumen microbial growth and thereby protein supply to the intestines. Collectively, therefore, supplements from these different broad groupings differ in their proportional contributions to the protein and energy nutrition of the animal, with these differences between supplement types often less than their relative composition would suggest (McLennan *et al*. 1995). Both supplement types also contribute to the mineral nutrition of the animal.

Numerous studies have been carried out investigating the growth responses to a wide range of supplements for growing cattle, including in northern Australia, but most have been restricted to only one or two levels of feeding (see Poppi and McLennan 1995) so that information on the marginal efficiency of supplement utilisation is generally not available. The primary objective of the present study was to compare growth response surfaces for supplements of different classes offered to cattle across a wide range of feeding levels. Supplements investigated were commercially available feed sources chosen to cover extremes of nutrient composition and digestion characteristics, particularly in relation to protein and energy supply. These included protein meals of medium (cottonseed meal) and low (fish meal) rumen degradability and energy substrates based on soluble sugars readily fermentable in the rumen (molasses), or on starch of low (sorghum) or high (barley) rumen degradability. Although these supplements have been variously investigated in the past, the uniqueness of the present study was that the comparison of response surfaces was carried out in a series of linked experiments, was made across a wide range in supplement intakes (up to 20 g/kg liveweight (W).day) exceeding those normally investigated, and used cattle and forages typical of those encountered during the dry seasons in northern Australia but also representative of tropical regions in general. Response surfaces so generated provide the basis for economic modelling and practical decision making.

Aside from the effects on animal growth, an important consideration in feeding supplements to ruminants in extensive grazing situations is to optimise utilisation of the base pasture, which usually represents the low cost component of the diet. Supplements can have either positive or negative (substitution) associative effects on forage intake, but generally with a stimulus in overall energy intake (Horn and McCollum 1987; Dixon and Stockdale 1999; Moore *et al*. 1999). Determining the effects of the different classes of supplement on forage intake was another important component of the present study.

This study compared the growth of steers, their intake and digestion of diets based on low-quality tropical hay, and various metabolic parameters for steers given a range of supplement types. This is the first of two papers describing the responses by cattle to supplement type and intake; the second paper investigates the effects of stage of maturity of the cattle on these responses (McLennan *et al*. 2017).

Materials and methods

Two sequential pen-feeding experiments of similar design were conducted at the Rocklea Animal Husbandry Research Farm, Brisbane and two sequential metabolism experiments were carried out at the Mt Cotton Research Farm, Brisbane. From here on, the experiments will be referred to as Pens1, Pens2, Metab1 and Metab2, respectively. All experiments were carried out under the supervision and with approval of the Department of Primary Industries (Queensland) Animal Ethics Committee.

Animals, diets, treatments and design

Pens1

One-hundred Brahman × Shorthorn (~75% *Bos indicus*) weaner steers ~6 months of age were used in the experiments. They were dipped to be free from ticks and drenched with oxfendazole (Systamex; Coopers Animal Health, Macquarie Park, NSW, Australia) at the recommended rate to reduce internal parasite burdens. The heaviest steers were drafted off for Pens1. During a 7-day equilibration period the steers were confined to outside pens in small groups and fed Callide Rhodes grass (*Chloris gayana*) hay *ad libitum* and small measured amounts of the supplements. These supplements were fed to accustom the steers to the experimental rations. At the end of this period the steers were weighed unfasted and fasted (24 h without food, 14 h without water) over successive days and allocated by stratified randomisation on the basis of this fasted W to experimental treatments and pens. A randomised block design was used. During a 63-day experimental period the steers received chaffed Rhodes grass hay *ad libitum* either unsupplemented (Control) or with supplements fed at 5, 10, 15 and 20 g/kg W.day (as-fed basis) of a sorghum mix (hereafter Sor diets), a barley mix (hereafter Bar diets) or a molasses mix (hereafter Mol diets). There were three Control steers in total and three replicates (steers) of each supplement treatment (type × level of feeding) making a total of 39 steers fed individually in pens. The initial unfasted W of the steers was 156.3 ± 4.91 (mean ± s.d.) kg.

The grain-based mixes consisted of (g/kg, as fed) 939 dry-rolled grain, 20 bentonite, 17.4 urea, 10 limestone, 10 molasses and 3.6 sulfate of ammonia. These ingredients were thoroughly mixed in a horizontal mixer before feeding. The molasses mix comprised (g/kg, as fed) 926 molasses, 28 urea, 27.4 added water, 9.3 salt (NaCl) and 9.3 di-calcium phosphate (170 g P/kg). These ingredients were mechanically mixed to dissolve all soluble components before feeding out.

Pens2

This experiment followed on immediately after Pens1. The remaining steers from the original draft grazed Rhodes grass pasture while Pens1 was underway. These steers were dipped and drenched (as above), confined to pens in small groups and fed Rhodes grass hay *ad libitum* with small measured amounts of the supplements being used, for 7 days. This hay was cut from the same area but at a different time to that used in Pens1. At the end of the equilibration period the steers were allocated to treatments in the manner described above. The initial unfasted W of the steers was 164.9 ± 5.70 kg. A randomised block design was used. Over a 63-day experimental period the steers received Rhodes grass hay *ad libitum* either unsupplemented (Control) or with supplements of cottonseed meal (hereafter CSM diets) or the molasses mix from Pens1, both fed at

(as-fed basis) 5, 10, 15 and 20 g/kg W.day or of fishmeal fed at 2, 4, 6 and 8 g/kg W.day (as fed). These experiments were carried out when the feeding of fishmeal in Australia was permissible. The CSM was fed as purchased commercially without additives. The lower intake levels of the fishmeal reflected previous experience with the amount cattle would consume. To increase palatability the daily treatment level of fishmeal was added to 1.5 kg of a molasses/water (1 : 2, w/w, as fed) mix and was fed as a slurry (hereafter FM diets). The molasses (Mol) treatments were the same as used in Pens1 and were repeated partly due to low intakes by steers in that experiment but also to provide reference to Pens1.

Metab1 and 2

Ten weaner steers from the same source as used in the pen experiments, and of mean starting W 146.8 ± 7.56 and 177.1 ± 11.51 kg, respectively, were used in two sequential metabolism experiments (Metab1 and Metab2) employing similar treatments and carried out concurrently with Pens1 and 2. Each experiment was divided into three periods (runs), each of 29 days' duration. In Metab1 the steers were fed Rhodes grass hay *ad libitum* from the same source as used in the pen experiments plus either the Sor or the Bar mix used in Pens1, fed at each of the five levels from Pens1, with one steer per feeding level (treatment) per run including one unsupplemented Control for each supplement type. The design was an incomplete Latin square (five treatments × three runs). For each run, steers were randomly allocated to treatments across supplement types according to the Latin square design with the restrictions that no steer received the same treatment more than once and that steers stayed on the same supplement type. Thus, over three runs each treatment was replicated three times. The same steers were re-randomised to treatment groups for Metab2 for which the basal Rhodes grass hay was similar, and procedures were the same, as in Metab1 but the supplements were CSM and Mol with supplement composition and intake levels those of Pens2.

Chemical composition of feedstuffs

The composition of the hay and supplements used in the pen and metabolism experiments is shown in Table 1. The hay and supplement mixes used in Metab1 and 2 aligned with those used in Pens1 and 2, respectively.

Table 1. Chemical composition of the feeds

The hay and supplements used in metabolism experiments were the same as those used for the respective pen experiments, and the molasses mix used in Pens2 was the same as that used in Pens1; OM, organic matter; N, nitrogen; NDF, neutral detergent fibre; ADF, acid detergent fibre; CF, crude fibre; EE, ether extract; –, not determined

Feed source	OM	N	EE	NDF	ADF	CF	Starch
				(g/kg DM)			
			Pens1				
Hay	902	9.2	15	713	372	–	–
Barley mix	951	32.1	22	–	–	42	554
Sorghum mix	955	31.1	31	–	–	19	681
Molasses mix	854	25.4	–	–	–	–	–
			Pens2				
Hay	900	10.5	15	699	387	–	–
Cottonseed meal	928	68.0	31	275	–	156	–
Fishmeal	829	115.2	97	59	–	–	–

Experimental procedures

Pens1

The steers were confined to individual outside pens, to which treatments were randomly allocated, and were fed hay and their allocation of supplements once daily in the morning (0800 hours). Hay was chaffed to a length of ~5–10 cm. In order to maintain *ad libitum* intake, the amount fed was adjusted each day following a bunk inspection with the aim of feeding ~15% more than was eaten on the previous day. The supplements were fed out in troughs separate from the hay. Intake of the grain mixes was slowly increased to treatment levels over the first week to reduce the risk of digestive problems, and the shortfall in intake in this week was made up over the following week. The supplement allocation for each day was added to any residue from the previous days except on the 1 day a week when residues were collected (see below). The amount of hay fed to the steers receiving the highest level of molasses mix (20 g/kg W.day) was restricted to slightly less than *ad libitum* from Week 3 to the end of the experiment in an effort to entice the steers to consume more molasses, as supplement intakes were below required rates on the higher intake treatments.

Residue feed (hay and supplement) was collected, weighed, subsampled and discarded once weekly. Subsamples of the hay and supplements fed were taken daily and kept for either DM determination or chemical analysis. Those kept for DM determination were pooled over 7 days and, except for the molasses, dried to constant weight at 100°C in a forced-draught oven. Weekly residue feed samples were treated in the same way. Subsamples of the molasses mix or its residue were weighed into scintillation tubes and mixed with a small quantity of distilled water. A weighed piece of filter paper was inserted into each tube and the tube was rotated so that the molasses mixture was absorbed onto the filter paper. This tube was then placed in a forced-draught oven at 100°C for 2 days and the DM content of the molasses was determined by weight change. Samples of other feeds kept for chemical analysis were pooled over ~35 days, dried at 60°C, ground through a 1-mm sieve and stored awaiting analysis. Chemical analysis of the molasses was carried out at the same frequency. Steers were weighed before feeding once weekly and a fasted weight, determined as described above, was recorded at the beginning and end of the experiment. Supplement allocations for each steer were individually adjusted weekly, on the basis of their most recent steer W, to maintain intakes at desired rates on a percentage of weight basis. Towards the end of the experimental period, faeces were collected from steers on the highest intake treatments for the Bar and Sor supplement groups and analysed for starch content. The faeces were collected from the floor of the pens at least four times over 24 h, taking care to avoid contamination from dirt, and then bulked for each steer, mixed thoroughly and stored at −18°C awaiting analysis.

Pens2

The procedures used in this experiment were the same as those used in Pens1, except where indicated below. As intakes of molasses for the highest molasses-fed group were generally below the desired levels, the hay allocation for this treatment group was slightly restricted from Week 2 onwards to encourage greater intake of the supplement.

Whereas residues of the hay, CSM and Mol supplements were handled in the same way as in Pens1, residues of the FM supplement were collected daily, weighed, dried to constant weight in a forced-draught oven at 60°C, and bulked over 7 days for each steer. After thorough mixing and subsampling, these bulked samples were ground through a 1-mm screen and stored awaiting analysis for N and organic matter (OM). This analysis was used to determine the proportion of the mixed residue that derived from fishmeal as opposed to molasses.

Metab1 and 2

Each run of the experiments comprised a 21-day preliminary period when steers were housed in individual outside concrete-floored pens to adjust them to their allocated rations and 8 days in metabolism crates indoors, including a 7-day collection period and a further day at the end for rumen and blood sampling. During the preliminary feeding periods, the steers were given access to a common dirt-floored yard for several hours each day to minimise the chance of feet soreness.

The general methods of feeding the hay and supplement have been described above (Pens1). Supplements were fed in separate trays to the hay to minimise mixing of the feed components and to allow differentiation of residues between feed sources. The amount of supplement offered each day was increased gradually over the first 14 days of the preliminary periods to allow steers to adapt between rations but final rations were fed over the last 7 days and during the collection period. Feed refusals, both hay and supplement, were collected each morning, weighed and bulked across days for each steer in each run. The total residue for each steer over the collection period was weighed and a subsample taken, dried in a forced-draft oven for 72 h at 65°C for DM determination, ground through a 1-mm screen and stored for analysis. Daily subsamples of the hay offered and of the grain and CSM supplements were also collected and bulked over the total collection period, and handled the same as feed residues. The molasses feed and residue samples were handled similarly but were dried as described above.

Faeces were collected and weighed daily and a 10% aliquot was stored frozen for each steer. At the end of the collection period, these daily samples were thawed and bulked for each steer, mixed thoroughly, subsampled and dried in the manner described above in order to determine total faecal DM output and subsequently OM digestibility (OMD). Another sample was freeze-dried and ground to 1 mm and stored for chemical analysis.

In Metab2 only, total urine was collected for estimation of microbial crude protein (MCP) synthesis (microbial crude protein synthesis, MPS). Urine was collected into 10% (v/v) sulfuric acid. The pH was measured regularly to ensure it remained below 3 and the quantity of acid adjusted if necessary. A 5-mL sample of the acidified urine was pipetted into a 50-mL graduated tube containing 1 mL of 1 mM allopurinol standard and made up to 50 mL with 0.1 M ammonium phosphate ($NH_4H_2PO_4$) buffer. The diluted sample was then frozen.

The steers remained in the metabolism cages and feeding continued as normal for 24 h after the completion of the 7-day collection period. Rumen fluid and blood samples were collected 3 h post-feeding on this final day and then rumen fluid was collected again the next morning (24 h post-feeding), just before re-feeding. Blood was collected from the jugular vein into a 10-mL tube containing lithium heparin and placed on ice. These samples were centrifuged at 700*g* for 10 min at 25°C and the resulting plasma was frozen awaiting analysis for urea-N content (PUN). Rumen fluid was collected from each steer using a stomach tube and vacuum pump and the sample was sieved through two layers of stocking before subsampling. A 4-mL subsample was added to 4 mL of 0.2 N HCl and frozen before analysis for ammonia-nitrogen (NH_3-N) concentration.

Laboratory analyses

Samples of the hay, grain mixes, cottonseed meal, fishmeal, feed residues and faeces were milled through a 1-mm screen before analysis. Ash content of all samples was determined by combusting ~2 g of oven-dried material in a muffle furnace at 550°C for 4 h, and OM was determined by difference from DM. The total N concentrations of samples were determined by a combustion method (Sweeney 1989) using an Elementar Rapid-N analyser (Elementar Analysensysteme, Hanau, Germany), which was calibrated using AR-grade aspartic acid. The fibre content was determined as follows: acid detergent fibre content by the method of Van Soest (1963), neutral detergent fibre content by the method of Van Soest and Wine (1967) and crude fibre content by standard AOAC (1975) procedures, all adapted for the Fibretec 2021 Fibrecap System (application sub-notes ASN 3801, 3804 and 3805, respectively), by FOSS TECATOR. A Soxhlet extraction process using hexane was employed to determine ether extract (EE; crude fat) content. The concentration of NH_3-N in rumen fluid was determined using a colourimetric method (Bolleter *et al.* 1961) whereas the PUN concentration was measured using a commercial enzymatic, colourimetric kit method (Trace Scientific, Melbourne, Vic., Australia; Tiffany *et al.* 1972). The starch in cereal grains and faeces was analysed by conversion to glucose using a two-step enzyme treatment and colourimetric determination of the glucose with a glucose oxidase/peroxidise reagent. All enzymes and reagents were supplied in kit form (Megazyme, provided by Deltagen, Melbourne, Vic., Australia). The enzymatic breakdown of the starch using a heat-stable α-amylase and amyloglucosidase is based on the procedure of McCleary *et al.* (1992).

Determination of purine derivative (PD) in urine was carried out using high-pressure liquid chromatography based on the method used by Balcells *et al.* (1992). Samples were thawed and filtered through 0.2-μm cellulose nitrate filters before analysis.

Estimation of microbial crude protein synthesis

The exogenous purine output (X; mmol/day) derived from the rumen microbial population was estimated as the total purine

excretion in urine (Y; mmol/day) less the endogenous contribution to the total, divided by a recovery factor. Verbic *et al*. (1990) suggested an endogenous purine contribution of 0.385 mmol/kg $W^{0.75}$.day for cattle and a recovery coefficient of 0.85 for absorbed purines in urinary PD. However, we used an endogenous value of 0.189 mmol/kg $W^{0.75}$.day, which was determined more recently by Bowen *et al*. (2006) for *Bos indicus* cattle. Thus, the equation used for calculation of endogenous purine supply was: $X = (Y - 0.189\ W^{0.75})/0.85$. Microbial N supply was then estimated (EMNS) from this calculated exogenous purine supply according to the equation of Chen and Gomes (1995) as: EMNS (g/day) = $70X/(0.83 \times 0.116 \times 1000) = 0.727\ X$, where 0.83 is the assumed digestibility of microbial purines and 0.116 the ratio of purine-N to total microbial-N. A factor of 6.25 was applied to convert EMNS to MPS (g/day).

In calculating the supply of rumen-degradable protein (RDP) the degradability of protein in hay and CSM was assumed, from nylon bag studies, to be 0.75 and 0.79 (S. McLennan, unpubl. data), respectively, and in molasses to be 1.0 (NRC 1996), with assumed passage rate through the rumen of 0.02/h.

Estimation of metabolisable energy intake and energy retention

The metabolisable energy intake (MEI) of steers was calculated as the product of the total DM intake, determined in the pen studies, and the energy density of the diet (M/D; MJ/kg DM), which was in turn derived using the equation of Freer *et al*. (2012) for mixed diets incorporating a supplement, i.e. M/D = 13.3 DMD + 23.4 EE – 1.71, where DMD is DM digestibility (g/g DM), determined from the metabolism studies, and EE is ether extract content of the diet (g/g DM). The DMD of Mol supplement in Metab2 was used to calculate MEI for the Mol treatments in both Pens1 and 2. Energy retention (ER) by the steers was calculated from W gain using equations from the Australian feeding standards (NRDR 2007) to first calculate the energy value of the gain. A standard reference weight of 600 kg was assumed for the steers. Energy retention was then regressed against MEI.

Statistical analyses

The results were analysed using Genstat (2015) to fit response curves of various shapes for different supplement types. In the majority of cases supplement intake was the independent variable but in others this was replaced by attributes such as digestible organic matter intake (DOMI) and MEI. Supplement intake for individual steers sometimes did not reach the intended levels, so measured intakes were used in the analyses. Data taken from the Control group of steers were used in establishing response curves with each supplement type within experiments. The Rep (replicate) and Pen effects were fitted first to the model followed by the linear and quadratic components for each supplement type. For each supplement, the significance of the quadratic effect was tested and removed from the model where it was non-significant. Where the quadratic effect was removed, the linear coefficient for each supplement type was similarly tested to determine whether the response to the supplement was significant. Equality between supplement response curves was tested where the response curves followed the same degree of polynomial. Where response curves for different supplement types were described by a different degree of polynomial, significant difference between treatments was assumed. Where the quadratic effect was significant, and it seemed biologically appropriate, an asymptotic curve was applied to replace the quadratic. Values for R^2 and residual standard deviation (RSD) were calculated relative to the model containing only Rep and Pen effects. For response curves where supplement intake was not the independent variable, and (X) values for the Control were not zero, for example DOMI and MEI, the average Control value was subtracted from the data to allow use of the methodology described above. Significant differences were determined as $P < 0.05$.

Results

Animal health and welfare

Despite consuming considerably high intakes of supplement, including grains, CSM and molasses, the steers showed no clinical evidence of ill-health including from acidosis and molasses toxicity across experiments.

Supplement intake

Across pen experiments, supplements associated with the Sor, Bar and CSM treatments were almost totally consumed on a daily basis whereas the maximum intake of the Mol supplements in both pen studies did not exceed ~12 g DM/kg W.day (or ~17.5 g as fed/kg W.day). Although fed at lower rates, problems were also encountered with acceptance of the FM supplement by steers even with the inclusion of molasses in the mix, and the maximum intake of the FM mixture averaged 5.5 g DM/kg W.day, for a maximum fishmeal intake of 4.1 g DM/kg W.day.

The Sor and Bar supplements in Metab1 and CSM in Metab2 were almost totally consumed but intake of Mol supplement in Metab2 was slightly below the intended rate and the maximum intake was 12 g DM/kg W.day. As supplements were formulated in amounts determined on an air-dry basis, the high moisture content of the molasses mix (~300 g/kg) contributed partly to its lower DM intake.

Starch in faeces

In Pens1, the average concentrations of starch in the faeces of steers on the highest level of supplementation in the Bar and Sor treatments were 61 and 252 g/kg DM, respectively.

Liveweight change

Control steers gained weight at just above maintenance in the two pen trials (0.08–0.15 kg/day). In Pens1, growth rates increased linearly with increasing intake of all supplement types where the rate of increase was 0.62, 0.45 and 0.33 kg/day per 10 g DM/kg W.day of Bar, Sor and Mol supplement intake, respectively (Table 2; Fig. 1*a*). Differences between response curves were significant in each case ($P < 0.05$). In Pens2, growth rate increased linearly at different rates ($P < 0.001$) of 1.73 and 0.33 kg/day per 10 g DM/kg W.day of FM and Mol supplement intake, respectively. By contrast, the growth

Table 2. Effect of supplement type (Supplement) and supplement intake (SI; g dry matter (DM)/kg liveweight (W).day) on the average daily gain and intake of hay and total DM in pen experiments, and on the digestibility of organic matter (OMD), intake of digestible organic matter (DOMI), concentrations of urea-nitrogen (urea-N) in blood plasma (3 h post-feeding) and of ammonia-nitrogen (NH_3-N) in rumen fluid (3 h and 0 h) in metabolism studies, and the effect of estimated metabolisable energy intake (MEI; kJ/kg $W^{0.75}$.day) on the estimated energy retention (ER) in pens, for steers fed low-quality hay *ad libitum*

Treatments included supplements based on sorghum mix, Sor; barley mix, Bar; molasses mix, Mol; cottonseed meal, CSM; and fishmeal/molasses mix, FM (see text for diet details). *P*-values are given for the linear (Lin.) and quadratic (Quad.) coefficients in the regression equations; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., non-significant ($P > 0.05$); RSD, residual standard deviation; treatment differences are discussed in the text. Where the quadratic relationship was significant, and it was considered biologically appropriate, an asymptotic function has been fitted to the data as represented by the included equations. The significance levels for the linear and quadratic tests of curvature are still presented but the R^2 and RSD apply to the asymptotic relationships

Y	Supplement	Equation	R^2	RSD	Lin.	Quad.
		Pens1 and Metab1				
Average daily gain (kg)	Sor	Y = 0.083 + 0.0447 SI	0.94	0.07	***	n.s.
	Bar	Y = 0.083 + 0.0618 SI	0.96	0.08	***	n.s.
	Mol	Y = 0.083 + 0.0329 SI	0.48	0.13	***	n.s.
Hay intake (g DM/kg W.day)	Sor	Y = 18.82 – 0.207 SI	0.50	1.34	**	n.s.
	Bar	Y = 18.82 – 0.506 SI	0.86	1.48	***	n.s.
	Mol	Y = 18.82 – 0.627 SI	0.65	1.47	***	n.s.
Total intake (g DM/kg W.day)	Sor	Y = 18.82 + 0.793 SI	0.93	1.34	***	n.s.
	Bar	Y = 18.82 + 0.494 SI	0.81	1.48	***	n.s.
	Mol	Y = 18.82 + 0.373 SI	0.50	1.47	***	n.s.
OMD (g/kg DM)	Sor	Y = 560.9 – 0.370 SI	0.87	9.52	n.s.	n.s.
	Bar	Y = 560.9 + 6.889 SI	0.89	26.39	***	n.s.
DOMI (g/kg W.day)	Sor	$Y = 14.57 – 5.23 (0.880^{SI})$	0.74	1.10	***	*
	Bar	Y = 9.43 + 0.497 SI	0.98	0.72	***	n.s.
ER (kJ/kg $W^{0.75}$.day)[A]	Sor	Y = –159.7 + 0.349 MEI	0.88	26.0	***	n.s.
	Bar	Y = –214.0 + 0.467 MEI	0.93	29.3	***	n.s.
	Mol	Y = –132.0 + 0.289 MEI	0.61	27.5	***	n.s.
Rumen NH_3-N, 3 h (mg/L)	Sor	Y = 42.23 + 12.51 SI	0.95	23.2	***	n.s.
	Bar	$Y = 253.7 – 222.9 (0.865^{SI})$	0.75	48.2	***	**
Rumen NH_3-N, 0 h (mg/L)	Sor	Y = 35.77 + 1.827 SI	0.57	14.5	*	n.s.
	Bar	Y = 35.77 + 1.537 SI	0.11	15.8	*	n.s.
Plasma urea-N, 3 h (mg/dL)	Sor	Y = 7.94 + 0.460 SI	0.72	1.9	***	n.s.
	Bar	Y = 7.94 + 0.530 SI	0.78	2.6	***	n.s.
		Pens2 and Metab2				
Average daily gain (kg)	CSM	$Y = 1.186 – 1.113 (0.744^{SI})$	0.89	0.15	***	***
	FM	Y = 0. 148 + 0.173 SI	0.87	0.15	***	n.s.
	Mol	Y = 0. 148 + 0.033 SI	0.67	0.13	**	n.s.
Hay intake (g DM/kg W.day)	CSM	Y = 23.82 – 0.339 SI	0.74	1.42	***	n.s.
	FM	Y = 23.82 – 0.837 SI	0.52	1.45	***	n.s.
	Mol	Y = 23.82 – 0.879 SI	0.68	2.46	***	n.s.
Total intake (g DM/kg W.day)	CSM	Y = 23.97 + 0.651 SI	0.93	1.42	***	n.s.
	FM	Y = 23.97 + 0.127 SI	0.08	1.47	n.s.	n.s.
	Mol	Y = 23.97 + 0.103 SI	0.05	2.45	n.s.	n.s.
OMD (g/kg DM)	CSM	Y = 578.1 + 0.88 SI	0.14	39.32	n.s.	n.s.
	Mol	Y = 578.1 + 6.11 SI	0.76	26.97	**	n.s.
DOMI (g/kg W.day)	CSM	Y = 10.52 + 0.422 SI	0.93	1.09	***	n.s.
	Mol	Y = 10.52 + 0.319 SI	0.85	1.02	***	n.s.
ER (kJ/kg $W^{0.75}$.day)[A]	CSM	$Y = 333.0 – 5277 (0.996^{MEI})$	0.85	45.3	***	***
	Mol	Y = –255.3 + 0.394 MEI	0.81	24.7	***	n.s.
Rumen NH_3-N, 3 h (mg/L)	CSM	Y = 30.23 + 14.09 SI	0.98	17.6	***	n.s.
	Mol	$Y = 146.2 – 125.2 (0.425^{SI})$	0.72	36.3	***	**
Rumen NH_3-N, 0 h (mg/L)	CSM	Y = 20.16 + 7.74 SI	0.92	18.1	***	n.s.
	Mol	Y = 20.16 – 0.340 SI	0.00	16.3	n.s.	n.s.
Plasma urea-N, 3 h (mg/dL)	CSM	Y = 5.25 + 1.495 SI	0.98	1.60	***	n.s.
	Mol	Y = 5.25 + 0.330 SI	0.46	2.80	*	n.s.

[A]These relationships, where MEI was the independent variable and the Control (X) values were not zero, include back-transformed values for the coefficients as the analyses were carried out on data after subtraction of the Control values.

response was quadratic to CSM intake and the fitted asymptotic curve indicated a steep response at low supplement intake levelling out at higher intakes for a peak (asymptote) weight gain of ~1.2 kg/day (Table 2; Fig. 1*b*).

Intake and digestibility

Intakes were determined in both the pen and metabolism experiments. As the response trends were similar for both forms of experiment but measurements in the pens were over longer duration (63 vs 7 days), only the pen results are presented. However, values for DOMI, which required a measure of OMD, are based on intakes and digestibilities from the metabolism studies.

The hay intake of the Control steers averaged 18.8 g DM/kg W.day in Pens1 but was 26% higher in Pens2 despite the hay being from a similar source. In both pen studies, increases in supplement intake were associated with linear reductions in hay intake and linear increases in total (hay + supplement) intake across supplement types (Table 2; Fig. 2). In Pens1 the reduction in hay intake was less ($P < 0.001$) for Sor than for Bar and Mol, which were not different ($P > 0.05$). The substitution rates, being the unit reduction in hay intake per unit intake of supplement, and as represented by the (negative) slope of the regression lines for hay intake, were 0.21 (Sor), 0.51 (Bar) and 0.63 (Mol). Conversely, total intake was increased more ($P < 0.001$) with Sor than with Bar and Mol (Table 2; Fig. 2*a*), which were again not different in effect. In Pens2 the reduction in hay intake with FM and Mol supplements was similar ($P > 0.05$; Table 2; Fig. 2*b*) and was considerably greater ($P < 0.001$) than for CSM. The substitution rates were 0.84 (FM), 0.88 (Mol) and 0.34 (CSM). Accordingly, the increase

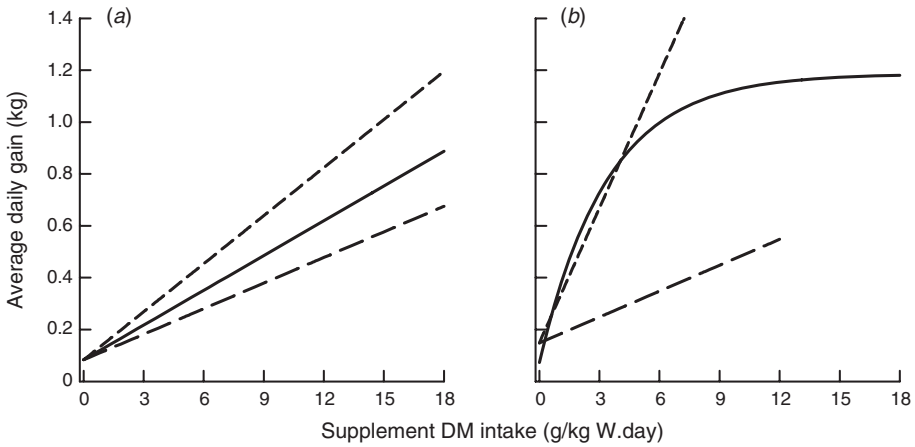

Fig. 1. Growth response relationships for steers fed supplements based on (*a*) sorghum (Sor; solid line), barley (Bar; short-dash) and molasses (Mol; long-dash) in Pens1 and (*b*) cottonseed meal (CSM; solid), fishmeal mix (FM; short-dash), and molasses (Mol; long-dash) in Pens2. The equations describing the relationships are given in Table 2.

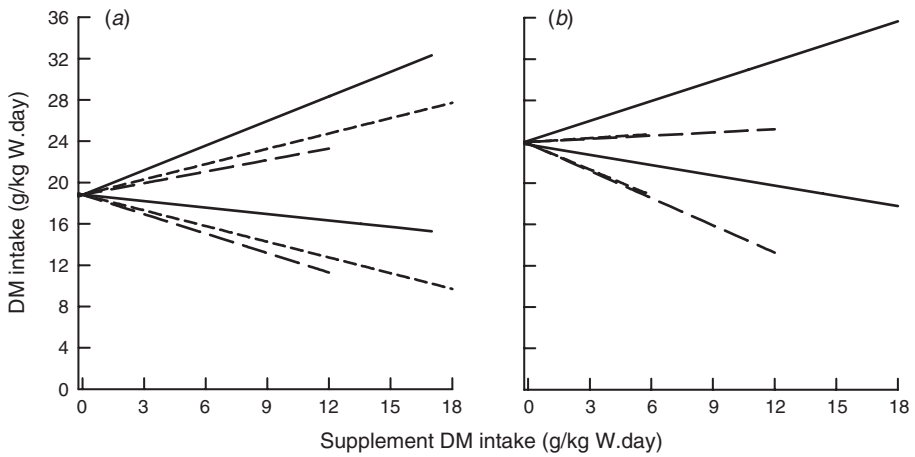

Fig. 2. Effect of supplement intake on the intake of hay (negative slopes) and total (hay + supplement; positive slopes) DM for steers fed supplements based on (*a*) sorghum (Sor; solid lines), barley (Bar; short-dash) and molasses (Mol; long-dash) in Pens1 and (*b*) cottonseed meal (CSM; solid), fishmeal mix (FM; short-dash) and molasses (Mol; long-dash) in Pens2. Note that FM and Mol lines overlay in (*b*). The equations describing the relationships are given in Table 2.

in total intake with increasing supplement intake was greater ($P < 0.001$) with CSM than with FM or Mol which had similar effect ($P > 0.05$) (Fig. 2*b*).

The OMD of the unsupplemented hay was 561 and 578 g/kg DM in Metab1 and 2, respectively. Increasing intakes of Bar but not Sor in Metab1 and of Mol but not CSM in Metab2 were associated with linear increases in OMD (Table 2). Consequently, the predicted OMD when the Bar supplement was consumed at the highest rate of ~17 g DM/kg W.day in Metab1 was 678 g/kg DM and with peak Mol intake of 13 g DM/kg W.day in Metab2 was 657 g/kg DM.

Across metabolism experiments, all supplement types were associated with increases in DOMI as supplement intake increased (Table 2). The DOMI increased linearly with increasing intake of Bar and quadratically with Sor in Metab1. When an asymptotic curve was fitted to the Sor data, DOMI approached a plateau at Sor intake of ~14.5 g DM/kg W.day. In Metab2 the responses were linear and not different ($P = 0.07$) for CSM and Mol treatments.

Metabolisable energy intake and energy retention

The estimated MEI increased linearly ($P < 0.05$) with increasing intake of all supplement types (relationships not shown). Estimated ER increased linearly with increasing MEI for all treatments in Pens1 but the slope of the regression relationship was greater ($P < 0.001$) for Bar compared with Sor and Mol treatments, which were not different ($P > 0.05$; Table 2; Fig. 3*a*). In Pens2, ER increased linearly with increasing MEI from the Mol diets but quadratically with CSM supplementation. An asymptotic function has been used to describe the relationship for the CSM treatment (Table 2; Fig. 3*b*).

Concentration of NH_3-N in rumen fluid

When assessed 3 h after feeding, the concentration of NH_3-N in the rumen increased linearly with increasing intake of Sor but quadratically with Bar in Metab1. Peak concentrations of NH_3-N of ~300 mg/L were reached with both supplements. An asymptotic function was fitted to the Bar data but the variability was relatively high about this curve ($R^2 = 0.75$, RSD = 48.2 mg/L; Table 2). At 24 h post-feeding (0 h) rumen NH_3-N concentrations were linearly related to intake of both Sor and Bar supplements, with no difference between them ($P > 0.05$), and the relationships indicated that an average supplement intake of ~9 g DM/kg W.day was required for NH_3-N concentration in the rumen fluid to exceed 50 mg/L.

At 3 h post-feeding, the concentration of NH_3-N in rumen fluid increased linearly with increasing intake of CSM supplement but quadratically with Mol in Metab2 (Table 2). With maximum intake of CSM of ~17 g DM/kg W.day, rumen NH_3-N concentration averaged 270 mg/L whereas with Mol the fitted asymptotic curve indicated that the concentration approached a plateau value of ~146 mg/L when intake of Mol supplement was ~8 g DM/kg W.day. This fitted asymptotic function indicated relatively high variability about the Mol data ($R^2 = 0.72$, RSD = 36.3 mg/L). At 24 h post-feeding (0 h) there was no effect of Mol on rumen NH_3-N concentration but a linear response to CSM, with NH_3-N concentration in the rumen fluid exceeding 50 mg/L when intake of CSM supplement exceeded 4 g DM/kg W.day.

Concentration of urea-N in blood plasma

Without supplement, the PUN concentration for the steers averaged between 5.2 and 7.9 mg/dL in the two metabolism trials but increased linearly with increasing intake of all supplement types. The response was similar for Sor and Bar ($P > 0.05$) treatments in Metab1 but in Metab2 the slope of the regression line was much greater for the CSM compared with Mol treatment ($P < 0.001$; see Table 2).

Microbial crude protein synthesis and efficiency of microbial crude protein synthesis

There were linear increases in the synthesis (MPS) and the efficiency of synthesis (EMPS) of MCP by steers in Metab2 with increasing consumption of supplement DM, with increasing

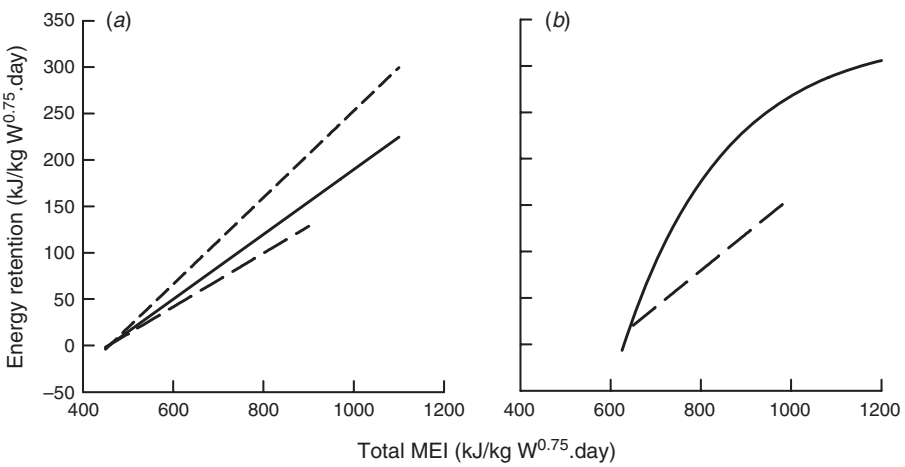

Fig. 3. Effect of estimated metabolisable energy intake (MEI) on the estimated energy retention of steers fed supplements based on (*a*) sorghum (Sor; solid lines), barley (Bar; short-dash) and molasses (Mol; long-dash) in Pens1 and (*b*) cottonseed meal (CSM; solid) and molasses (Mol; long-dash) in Pens2. The equations describing the relationships are given in Table 2.

Table 3. Effect of supplement type (Supplement) and of intakes of supplement [SI; g DM/kg liveweight (W).day], total digestible organic matter (DOMI; g/kg W.day) and total rumen-degradable protein (RDPI; g/kg W.day), and of the ratio of RDPI/DOMI (RDPOM; g/kg), on microbial crude protein (MCP) synthesis (MPS) and the efficiency of MPS (EMPS) for steers fed low-quality hay *ad libitum* in Metab2

Treatments included supplements based on cottonseed meal, CSM and a molasses mix, Mol (see text for diet details). All relationships, except those including SI as the independent variable, used back-transformed values for the linear coefficients as the analyses were carried out on data after subtraction of the Control (X) values, which were not zero. *P*-values are given for the linear (Lin.) coefficient in the regression equations only as the quadratic functions were non-significant in each case; ***, $P < 0.001$; RSD, residual standard deviation; treatment differences are discussed in the text

Y	Supplement	Equation	R^2	RSD	Lin.
MPS (g/kg W.day)	CSM	Y = 0.947 + 0.105 SI	0.93	0.23	***
	Mol	Y = 0.947 + 0.105 SI	0.95	0.19	***
	CSM	Y = –1.510 + 0.239 DOMI	0.90	0.29	***
	Mol	Y = –1.914 + 0.280 DOMI	0.91	0.23	***
	CSM	Y = 0.599 + 0.343 RDPI	0.94	0.21	***
	Mol	Y = 0.264 + 0.717 RDPI	0.93	0.21	***
	CSM	Y = 0.326 + 0.0064 RDPOM	0.91	0.27	***
	Mol	Y = –0.264 + 0.0128 RDPOM	0.94	0.20	***
EMPS (g MCP/kg DOM)	CSM	Y = 91.39 + 4.078 SI	0.79	16.7	***
	Mol	Y = 91.39 + 5.558 SI	0.93	11.4	***
	CSM	Y = 0.213 + 8.97 DOMI	0.70	19.9	***
	Mol	Y = –46.69 + 13.78 DOMI	0.82	18.0	***
	CSM	Y = 78.24 + 13.22 RDPI	0.79	16.5	***
	Mol	Y = 56.88 + 37.03 RDPI	0.88	14.6	***
	CSM	Y = 66.86 + 0.250 RDPOM	0.78	17.1	***
	Mol	Y = 27.55 + 0.673 RDPOM	0.93	11.4	***

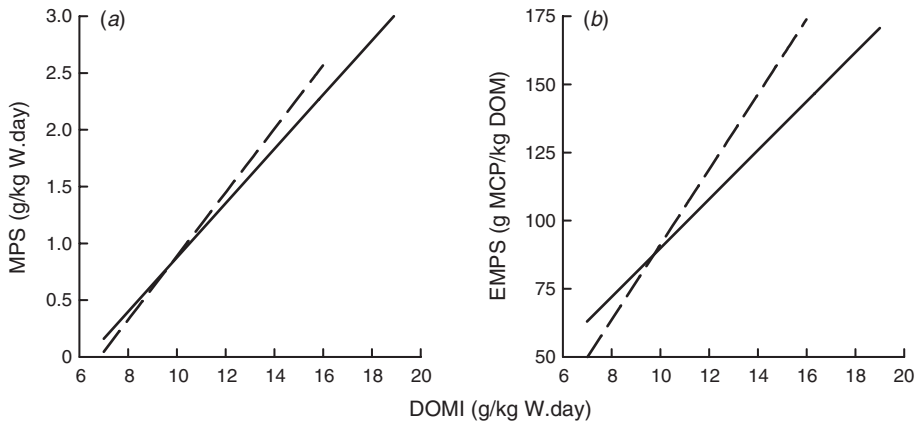

Fig. 4. Effect of digestible organic matter (DOM) intake (DOMI) on (*a*) microbial crude protein (MCP) synthesis (MPS) and (*b*) the efficiency of MPS (EMPS) for steers receiving supplements based on cottonseed meal (CSM; solid line) or molasses (Mol; dashed line) in Metab2. The equations describing the relationships are given in Table 3.

intakes of DOM and of RDP, and with increasing ratio of RDP : DOM in the total diet (Table 3). The responses in MPS were similar ($P > 0.05$) for CSM and Mol treatments when compared relative to absolute supplement intake (g DM/kg W.day; results not illustrated) and to the total DOMI (Fig. 4) but the response was higher (greater slope) with Mol compared with CSM when compared on the basis of RDP intake ($P < 0.001$; results not illustrated) or RDP : DOM ratio in the diet ($P < 0.001$; Fig. 5). The same effects applied with EMPS except that the response was slightly greater ($P = 0.04$) for Mol than CSM when compared on a DOMI basis (Figs 4 and 5) and tended to be greater on a supplement DM intake basis ($P = 0.05$). The equations describing these relationships are given in Table 3.

Discussion

The aim of this study was to compare the response relationships to a variety of supplement types with young steers given a low-quality tropical forage representative of that encountered by cattle grazing during the dry season in the seasonally dry tropics of Australia. Supplements were chosen to cover extremes of nutrient composition and digestion characteristics,

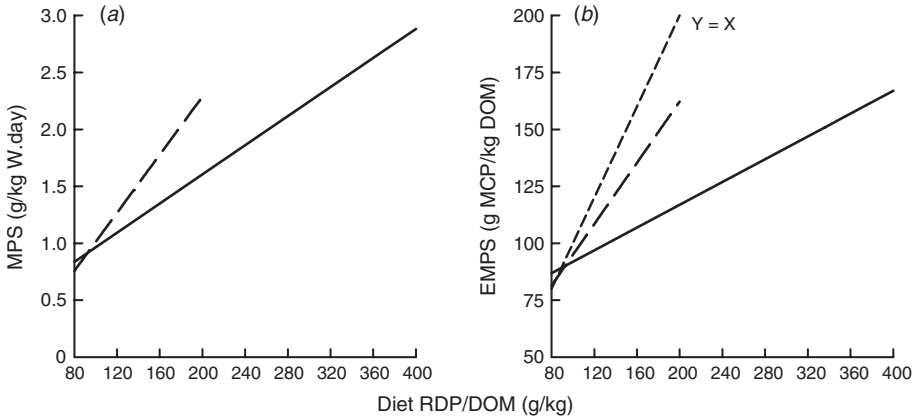

Fig. 5. Effect of the ratio of rumen-degradable protein (RDP) to digestible organic matter (DOM) in the diet on (*a*) microbial crude protein (MCP) synthesis (MPS) and (*b*) the efficiency of MPS (EMPS) for steers receiving supplements based on cottonseed meal (CSM; solid line) or molasses (Mol; long-dashed line) in Metab2. The Y = X line is shown as a short-dashed line in (*b*). The equations describing the relationships are given in Table 3.

particularly in relation to protein and energy supply. In view of the scope of supplement types investigated two experiments were carried out with standardised experimental procedures, with cattle from the same source and with common basal forage diets, which elicited similar growth rates in unsupplemented steers (0.08 and 0.15 kg/day).

Growth responses

The low growth rates of Control steers in the pen studies (0.08–0.15 kg/day) were consistent with the low quality of the tropical hay fed and are also typical of the regularly experienced low performance of cattle grazing unimproved native pastures during the dry season across much of northern Australia. Responses to the addition of nutrients from supplements were thus not surprising but these responses varied widely with supplement type. Quantitatively, the response surfaces seem divisible into two main categories: supplements with high protein content, i.e. CSM and FM, and those with lower protein but relatively high energy content, i.e. Bar, Sor and Mol, often referred to as energy sources. The growth response to increasing CSM intake was distinctly curvilinear with growth increments described by the law of diminishing returns. This pattern of steep initial response followed by more gradual increase at higher intakes has been exhibited in other similar studies using the combination of young cattle, low-quality basal forage and a wide range in CSM intakes (upper limits of 8–16 g DM/kg W.day) (Dolberg and Finlayson 1995; McLennan and Poppi 1995; (liveweights provided by S. McLennan, unpubl. data); McLennan *et al*. 2017). From a compilation of research findings, Leng (2004) proposed that for young cattle given basal diets of low-quality forage, a log-linear relationship best described the W response to additional protein from protein meals, although he proposed that for practical purposes this could logically be considered as two independent linear relationships, one with a high slope representing low intakes and the other at a lesser slope for higher intakes of supplement. Although Smith and Warren (1986*a*, 1986*b*) conversely reported linear responses to CSM by steers grazing low-protein pastures across the full range of intakes, the maximum CSM intakes were just 3.6 and 3.2 g DM/kg W.day, respectively, and thus would have fallen within the steep section of our plotted response curve.

We propose an asymptotic, rather than log-linear, relationship (see Fig. 1) as it uses three compared with two parameters to define the response curve and, when tested with our data, it provided a higher R^2 value than the corresponding log-linear version. It is also logical that growth rate will eventually reach a plateau value, which in our study occurred at ~1.2 kg/day when CSM intake exceeded ~13 g DM/kg W.day. However, from a practical viewpoint, the most cost-effective response to CSM is likely to occur within the steep section of the response curve or up to an intake of ~5 g DM/kg W.day based on our results. Within this section of the response surface, the conversion rate was equivalent to ~0.9 : 1 added gain per unit of CSM DM (0.8 : 1, as fed) for a 200-kg steer. McCollum and Horn (1990) have reported corresponding conversion rates with protein meals as high as 2 : 1 for ruminants given access to low-quality forages but these rates would have been at the low end of the intake scale and would most likely have incorporated a substantial stimulus in forage intake.

Although fishmeal can no longer be fed to ruminants in Australia its inclusion here allows an examination of the effects of a protein source which generally has only low degradability in the rumen, especially compared with CSM (Madsen and Hvelplund 1994), but still provides amino acids to the animal post-ruminally. That the curvilinear response pattern to CSM was not replicated with the FM supplement, but instead was linear, is probably an artefact of the low range of intakes spanned with FM supplement, which also included molasses in the mix. The proportion of fishmeal in the FM supplement averaged 63% (range 49–75%) so the range of fishmeal intakes was only 1.2–4.1 g DM/kg W.day. The FM response curve aligns with the steep section of the CSM response curve and corresponds, for a 200-kg steer, to a conversion rate of 1.0 : 1 added gain per unit of FM (DM) for the total mixed supplement, or an average 1.4 : 1 for the fishmeal

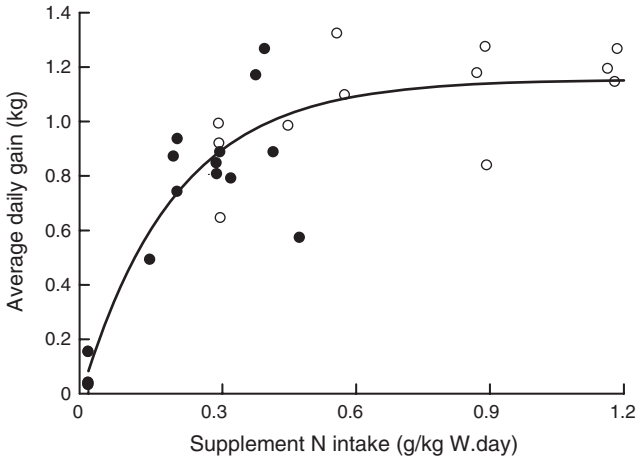

Fig. 6. Effect of the nitrogen (N) intake from cottonseed meal (CSM; open circles) and fishmeal/molasses (FM; closed circles) on the growth rate of steers in Pens2. The equation describing the relationship is: $Y = 1.155 - 1.072\ (0.0087^{X})$; $R^2 = 0.77$, RSD = 0.17, $P < 0.001$.

(DM) component only, ignoring any contribution from the molasses.

Much of the difference between the CSM and FM supplements can be attributed to differences in protein content, as indicated in Fig. 6, where the growth responses relative to supplement-N intake seem to fit a common response curve albeit that the CSM intakes were over a much wider range. Another contributing factor would be the previously mentioned differences in rumen degradability of the protein meals, being much higher in CSM than for fishmeal. On the basis of the asymptotic relationship illustrated in Fig. 6 the growth response approached a plateau when the supplement-N intake was ~0.8 g/kg W.day. This curve is consistent with the concept proposed by Black and Griffiths (1975) that ruminant production increases with increasing N intake until energy becomes limiting, whereupon further increases in production with N input require higher energy intake. That the plateau occurred at such a high growth rate for steers on a low-quality forage suggests that N and not energy was the primary limiting nutrient for the steers.

The responses to the energy sources fed in Pens1 were linear with the response rate, as depicted by the slope of the regression line, declining in order of: Bar (slope 0.062), Sor (0.045) and Mol (0.033). Expressed in practical terms, these response rates translated to respective conversion rates of 0.31, 0.22 and 0.16 g additional gain/g supplement DM intake based on a steer W of 200 kg, considerably less than that for the protein meals in Pens2 at low to medium intakes. In fact, considered across experiments, only when intakes reached the highest level of ~17 g/kg W.day was there apparent convergence of response curves for the Bar and CSM treatments. In a compilation of data from published experiments involving a wide range of supplements based largely on energy sources Poppi *et al*. (1999) also deduced a linear response relationship across a wide range of intakes.

The higher feeding value of barley compared with sorghum has been long recognised (e.g. Saba *et al*. 1964) although the disparity between the grains is influenced by the varieties used, especially with sorghum, as well as the intensity of processing, as sorghum appears to respond more than barley to processes such as steam-flaking (Waldo 1973; Rooney and Pflugfelder 1986). Herrera-Saldana *et al*. (1990) assessed the ruminal starch digestibility *in situ* of barley and sorghum, milled to 1 mm, to be 90% and 49%, respectively, whereas Huntington (1997) determined that in the dry-rolled form corresponding values for starch digestibility of both grains in the rumen of cattle were 81% and 60%, respectively. It is apparent that in the present study starch digestibility in sorghum could have been even lower than these values suggest given the very high concentration of starch in the faeces (up to 252 g/kg DM). This indicates that a low digestibility source of sorghum was chosen for the experiments and/or that processing of the grain was inadequate resulting in coarse particle size (Waldo 1973), and the results of the current comparisons should be evaluated accordingly. Nonetheless, evidence from the literature of the higher energy value of barley compared with sorghum in practical feeding rations for cattle is compelling. Owens *et al*. (1997) analysed extensive data from North American feeding trials and calculated bodyweight-adjusted ME values for (dry-rolled) barley and sorghum of 14.9 and 12.1 MJ/kg DM, respectively.

The inferior performance of molasses as an energy source relative to grains has been reported previously (Pate 1983). Gulbransen (1985) showed that at the low intakes used for survival feeding of cattle (up to 3 kg/day), molasses had ~85% of the energy value of grain sorghum on a DM basis. Furthermore, under *ad libitum* production feeding, he reported substantial increases in the feed intake and growth rate of cattle as sorghum progressively replaced molasses in the ration. In studies with finishing steers, Lofgreen and Otagaki (1960) showed that at feeding rates of 10% of the DM in the ration, molasses had a relatively high net energy value but as its proportion increased to 25–40%, the net energy value was almost halved. Subsequently, Lofgreen (1965) showed that at feeding levels of 15% of total DM and less, molasses had a net energy value of 74% of that of barley. In our study the growth response rate to Mol was ~73% and 53%, on average, of that for Sor and Bar, respectively, as indicated by the slope of the regression relationships.

These response relationships clearly demonstrate the superiority of protein meals, relative to energy sources, for increasing growth rate of young steers given low-quality forage-based diets when supplement intakes are low, but at higher intakes there appears a tendency for convergence of the response curves, at least for the Bar and CSM supplements. These results strongly indicate an initial deficiency of protein, presumably for both the rumen and total animal, but subsequent increases in growth rates appear dependent on increasing energy intake in general, balanced with protein supply. There were differences between energy sources which can be exploited practically when combined with knowledge of their respective costs.

Intake and digestibility

Provision of supplements to cattle fed low-quality forages has variable effects on forage intake, as has been extensively reviewed in the past (e.g. Horn and McCollum 1987; Caton and Dhuyvetter 1997; Dixon and Stockdale 1999; Moore *et al*.

1999; NRDR 2007). Both positive (forage intake stimulus) and negative (intake substitution) associative effects have been reported, dependent on a whole range of factors but particularly on the type and composition of the supplement and its level of dietary inclusion. However, in our study, all supplement types were associated with a stepwise linear reduction in hay intake, but a corresponding linear increase in total DM intake, although the amplitude of these effects varied widely with supplement type.

The reasons for the lower substitution rate (the unit reduction in forage intake per unit intake of supplement) with Sor (0.21) compared with Bar or Mol (0.51 and 0.63) in Pens1 could be several-fold. One explanation is provided in the conceptual model of Weston (1996), which attributes intake regulation by ruminants to an interplay between the energy deficit, defined as the difference between the capacity of the animal to use energy in its current physiological state and the useful dietary energy intake, and the rumen digesta load, which is a function of the animal's capacity to clear digesta from the rumen. An example of this was provided in the research of Gherardi and Black (1989) who showed that as the amount of nutrients infused into the abomasum of sheep increased, thereby reducing the energy deficit, the voluntary intake of wheaten hay OM declined linearly, which was associated with a decline in rumen digesta contents. The higher substitution rate with Bar compared with Sor is consistent with its higher ME content, as indicated above, and thus lower energy deficit relative to Sor.

However, this does not explain the higher substitution with Mol compared with Sor given the evidence above of lower ME content of molasses relative to the grains. A possible contributing factor is an insufficiency in supply of RDP relative to fermentable energy in the diet, the importance of which has been previously emphasised, especially in relation to grain-based supplements (Lusby and Wagner 1986; Chase and Hibberd 1987; Horn and McCollum 1987; Bodine and Purvis 2003). Although we provided a set proportion of urea in the grain and molasses mixes, calculated to be in excess of that required for fermentation of just the supplement component in the rumen, RDN may still have been limiting in the total diet when supplement intake rates were low and the proportion of forage of low CP in the diet high. This effect would have been more pronounced with the Bar and Mol supplements, which were associated with linear increases in total diet OMD, presumably resulting from the higher digestibility of the supplements relative to the hay they replaced. By contrast, Sor supplement did not increase OMD, which, coupled with the high concentration of starch in faeces of the steers on the Sor diet, suggests low digestion of sorghum in the total tract and presumably therefore also in the rumen. It could be surmised, therefore, that proportionately higher concentrations of RDN were required with Bar and Mol than with Sor, making the former diets more susceptible to any shortfall in RDN supply.

In their reviews, McCollum and Horn (1990) and Owens *et al.* (1991) deduced that forage intake was usually increased with protein supplementation but this was dependent on the protein content of the basal forage. However, despite the relatively low quality of the forages used in our studies (~10 g N/kg DM) there was no increase in forage intake with even the lowest intakes of CSM and FM supplements. Evidence has been provided for both an increase (Hunter and Siebert 1980; Church and Santos 1981; Hennessy *et al.* 1983; McCollum and Galyean 1985; McLennan *et al.* 2017) and decrease (Perdok and Leng 1990; Dolberg and Finlayson 1995) in intake of low-quality forage with protein supplementation, the diversity of effects probably being largely dose-related. In studies involving infusion of graded levels of casein into the rumen of cattle fed low-quality forage, forage consumption changed in a quadratic fashion, that is, an initial increase followed by a plateau or decline (Köster *et al.* 1996; Klevesahl *et al.* 2003; Wickersham *et al.* 2004). This suggests an initial correction of a ruminal-N deficiency, notwithstanding that other nutrients provided by the protein sources such as amino acids and branched-chain volatile fatty acids could also be involved, leading to increased flow of MCP to the duodenum (Hunter and Siebert 1980; Köster *et al.* 1996). Failure to isolate any positive effects on hay intake from protein feeding in our study, at least with the CSM, may also be an artefact of the high levels of feeding and absence of supplement increments between zero and 5 g/kg W.day. For instance, in the casein infusion experiments described above, the range in estimated intakes of digestible intake protein (DIP) from the casein were 0–1.2 (Köster *et al.* 1996), 0–1.8 (Klevesahl *et al.* 2003) and 0–1.4 g/kg W.day (Wickersham *et al.* 2004), with 4–6 supplement increments within these ranges, compared with an estimated DIP range of 0–4.2 and a first increment of feeding of 1.1 g/kg W.day in the present study (assume 24.2% DIP in CSM; NRC 1996).

The same line of reasoning does not seem to apply to the FM supplement for which intakes were much lower compared with CSM but the reduction in hay intake greater. The rumen degradability of protein in the fishmeal was not determined in our study but in a parallel study using fishmeal from the same source the degradability of CP was determined *in sacco* to be 37% for an assumed fractional outflow rate of 0.02/h (M. J. Bolam, unpubl. data). The high growth response to FM, which was higher than for CSM over the same intake range, coupled with the reduction in hay intake in our study suggests that the protein from the FM supplement largely escaped digestion in the rumen, contributing to a deficiency of RDN. However, other workers have shown that protein infused post-ruminally or protein protected from rumen degradation by formaldehyde can increase intake of low-quality forage by cattle (Hennessy and Williamson 1990; Wickersham *et al.* 2004). It is unlikely that the small inclusion of molasses in the FM supplement (average 1.4 g/kg W.day, or 37% of DM in FM) would have contributed appreciably to the substitution effect. The reasons for this reduction in forage intake remain unclear.

In summary, although all supplements were associated with linear reductions in forage intake, the mechanisms involved for different types may have varied. Failure to include intake levels less than 5 g/kg W.day with most supplements, except FM, possibly concealed any positive effects on hay intake of small supplement intakes. Forage intake reduction otherwise was likely associated with effects of one or both of an energy deficit and a deficiency in RDN to utilise the fermentable carbohydrate in the rumen. The importance of providing adequate RDN for the total diet for optimum forage utilisation is highlighted.

Energy intake and utilisation

Despite these reductions in forage intake, the DOMI and estimated MEI (results not shown) increased with intake of all supplements demonstrating that the reduced energy contribution from the hay was more than completely compensated by that provided in the supplements. These effects stemmed from the higher total DM intakes with intake of all supplements coupled, in the case of Bar and Mol but not Sor or CSM, with higher dietary OMD. The derived relationships between MEI and ER (see Fig. 3) were linear for the various energy sources, suggesting for each supplement a constant coefficient of utilisation of ME for growth, or 'virtual' k_g value, represented by the slope of the regression line. This cannot be considered a true k_g as the increases in MEI were not achieved at constant dietary M/D, which instead increased with increasing supplement intake in each case. Nevertheless, the higher 'estimated' k_g for Bar (0.47) compared with Mol and Sor (0.32 average), indicates higher efficiency of use of energy from this source and is consistent with the higher growth rates for steers on the Bar treatment relative to other energy sources in the absence of increases in total DM intake. The estimated maintenance requirement of these steers (zero ER) was, on average, 457 kJ/kg $W^{0.75}$.day in Pens1, comparable to that of 417 kJ/kg $W^{0.75}$.day from the subsequent study in this series with similar young cattle (McLennan *et al*. 2017).

In contrast to the relationships with energy sources in Pens1 and 2, there was deviation from linearity with the CSM diets whereby the apparent efficiency of utilisation of energy declined with increasing CSM intake (see Fig. 3). Nevertheless, the interrelationship between ER and MEI increased much more steeply for the CSM than for Mol in Pens2, and apparently than that for the other energy sources in Pens1, at low to medium CSM intakes. This declining slope at high intakes of CSM could be a function of increased outflow rate from the rumen (AFRC 1993) or alternatively could reflect the high energetic cost of excreting urea in excess of that required for bacterial or tissue needs (see NRC 1989), as indicated by the associated high PUN concentrations for this treatment. Black and Griffiths (1975) proposed a theoretical interrelationship of protein intake with energy and protein retained whereby at fixed energy intake, protein retention initially increased linearly as protein intake increased but then reached a plateau when energy became limiting. It is possible, therefore, that the decline in ER at high CSM intakes indicated that energy had become limiting for further protein deposition and associated animal growth. In summary, the energy utilisation trends displayed in these studies with tropical diets and cattle are consistent with the general concepts espoused in the various feeding standards.

Rumen and blood metabolites and microbial crude protein synthesis

Without supplement, the concentrations of NH_3-N in the rumen fluid of steers (20–42 mg/L) were lower than the 50 mg/L suggested by Satter and Slyter (1974) as the threshold below which microbial growth was limited. These concentrations were consistent with the low protein content of the tropical grass hay fed. Inclusion of urea in any of the energy supplements markedly increased the concentration of NH_3-N in the rumen fluid 3 h after feeding although the effect was highly variable, apparently reflecting the variable rate of intake of supplement and the abrupt response in NH_3-N concentration in the rumen to even small intakes of urea. These elevated NH_3-N concentrations were obviously only temporarily sustained after intake of the urea-inclusive supplements was completed, usually in the first few hours after presentation, as concentrations 24 h after feeding (0 h) were low and in the case of Mol not different to that of the Controls. Rapid uptake of this ammonia by rumen microbes, in the presence of readily fermentable energy sources, would also contribute to the depletion of ammonia in the rumen.

By contrast there was a strong linear relationship between intake of CSM and rumen NH_3-N concentration at both sampling times, indicative of a sustained breakdown of protein in the rumen with this protein meal thereby providing more continuous supply of N substrates for microbial growth. McCollum and Galyean (1985) reported a similar sustained elevation of rumen ammonia concentrations after the feeding of CSM to steers fed low-quality prairie hay. In support, the effective degradability of protein in CSM from similar sources in Queensland, determined *in sacco* and calculated using a passage rate of 0.02/h, has been variously estimated at 0.77 (Moss *et al*. 1998) and 0.79 (S. McLennan, unpubl. data), compared with 0.73 in a European ringtest (Madsen and Hvelplund 1994). These values confirm a relatively high degradability of protein in the rumen but still with some protein transferred undegraded to the intestines.

The PUN concentrations provide a more pragmatic indication of the N status of the steers, being less responsive to transient supplement intake effects and reflecting the end-point of the various processes of intake, uptake, absorption, recycling and excretion of N on a daily basis. Although all supplements increased PUN concentration measured 3 h after supplement feeding, the largest response was to CSM, which resulted in very high concentrations of PUN (~30 mg/dL) at the highest supplement intakes, which would require substantial energy expenditure for excretion of excess urea, as alluded to above.

Although only two of the diets were assessed for their effects on MPS in the rumen these diets included the extremes of a highly fermentable soluble sugar source (Mol) and a protein source with moderately high rumen degradability (CSM). A collation of values from various feeding standards (Poppi *et al*. 1999) suggests that EMPS from forages should generally fall within the range of 130–170 g MCP/kg DOM when RDN is not limiting, although lower values could be expected with tropical grasses with low CP content (see also NRDR 2007). The value of 91 g MCP/kg DOM we estimated for EMPS with steers given unsupplemented Rhodes grass hay is consistent with that of Tuyen *et al*. (2015) for steers given pangola grass (*Digitaria eriantha*) hay (83 g MCP/kg DOM) and with those of Bowen (2003) of 26–90 g MCP/kg DOM for cattle grazing tropical grass pastures, these values all seemingly related directly to RDN supply. Although EMPS was increased linearly with increasing intake of both CSM and Mol, the lower value in the feeding standards range of 130 g MCP/kg DOM was not achieved until supplement intakes exceeded 9.4 and 6.9 g DM/kg W.day, respectively. Tuyen *et al*. (2015) recorded a quadratic increase in EMPS with increasing proportion of molasses and urea in a

mixed diet of molasses, pangola grass and urea, with maximum EMPS of 138 g MCP/kg DOM when molasses and urea comprised 75% of the diet, but they estimated that the EMPS attributable to the molasses and urea component of the diets was 166 g MCP/kg DOM. By comparison, in our study estimated EMPS was 160 g MCP/kg DOM when intake of the molasses mix peaked at 12.3 g DM/kg W.day and constituted ~52% of total DM intake. This confirmed the high potential for molasses-based supplements to increase EMPS as a consequence of high rumen-fermentable ME content.

The expression of EMPS in terms of RDN and energy (DOM) supply reflects the importance of, and interrelationship between, these elements in determining MPS. In regressing EMPS against intakes of both DOM and RDP (not illustrated), or the ratio of these factors, the closest agreement between supplement types seemed to be with DOMI on the independent axis although Mol provided a slightly higher increase in EMPS per unit of DOM than CSM ($P = 0.04$; see Fig. 4). This difference may reflect the higher proportional OM digestion in the rumen with Mol compared with CSM diets (Tuyen *et al*. 2015) leading to lower relative estimates of EMPS for CSM compared with Mol diets where DOM referred to whole-track digestion. Closer agreement between diets would have occurred if MPS was expressed relative to the OM digested only in the rumen, i.e. to fermentable ME. Rumen digestion was not measured in our experiments but could be inferred, as discussed above. Fig. 5 shows the y = x relationship between EMPS and the RDP : DOM ratio illustrating that at low levels of supplementation, EMPS was closely related to RDP : DOM supply but that as this ratio increased there was increasingly less capture of the RDP by the microbes. However, this effect was diet dependent; with the Mol diets there was reasonably close agreement between the ratios of MPS : DOM (EMPS) and diet RDP : DOM whereas with the CSM diets there was a very large disparity between the two ratios, indicating an excess of RDP relative to DOM and thereby reinforcing the fact that EMPS was most closely aligned with DOMI when RDN meets minimum requirements. These results do emphasise though the importance of providing sufficient RDN to optimise utilisation of the supplement but also the need to provide additional RDN to ensure that N is not limiting in the rumen for fermentation of the forage component of the diet. Consequently, the requirements for RDN will change with the proportion of supplement in the total diet and their respective protein and fermentable energy characteristics but it is important that sufficient RDN is provided to ensure no shortage for fermentation of the total diet, not just the supplement. Practically it is difficult to balance the total diet for RDN supply when supplement intakes are low, and forage intake high, because of the risks of urea toxicity. Using a RDP source like CSM overcomes this problem.

Conclusions

The establishment of these response curves comparing a range of supplement types has provided strategic information about the nutritional limitations to the growth of young cattle given low-quality tropical forages, of particular relevance to cattle grazing dry season native pasture in northern Australia. With the inclusion of information on supplement costs, they further provide a framework upon which practical decisions can be based to meet growth targets under commercial grazing conditions.

The notable result was the steep growth response by steers to small amounts of protein meal, reinforcing the assertion that protein is the primary deficiency for cattle growth on these senesced tropical forages (Poppi and McLennan 1995) and that major improvements in animal performance can result from protein supplementation. In the case of cottonseed meal, this high growth response apparently derived from several factors, viz., increased supply of metabolisable protein for intestinal absorption in the form of both microbial protein and undegraded dietary protein, increased energy intake, and perhaps increased utilisation of ME by virtue of this increased supply of MP and an increase in the MP : ME ratio (Gill *et al*. 1984). On face value it would appear that the most cost-effective strategy would be to exploit this high growth response to protein meals at low intake rates. This approach is also consistent with optimising use of the pasture, the low cost component of the diet. Where higher growth rates are targeted, thereby demanding higher supplement intakes, the convergence of the growth response curves for protein meals and energy sources, and the generally lower cost of the latter, may result in the energy sources being the supplement of choice on a cost/benefit basis. However, it is conceivable that a combination of supplement types will often be most appropriate to optimise both animal performance and cost. Fig. 1 provides a summary of the response curves, which can be used practically to decide on the level of supplementation for a single supplement type. Balancing RDP : DOM for the whole diet is an accepted principle but difficult to achieve at low supplement intakes with just urea, and although some inclusion of protein meals is safer to use the final decision will be based on the response and cost. Nutrient-unbalanced diets can be just as or more cost-effective as nutrient-balanced diets.

The results of these studies apply primarily to young, growing steers. Because nutrient requirements change with increasing maturity of the animal (Black and Griffiths 1975; Poppi 1990) it would be expected that the response surfaces will also change for older cattle. An examination of the effects of stage of maturity of cattle on the responses to various supplement types is the subject of a second paper of this series (McLennan *et al*. 2017).

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