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Change in metabolite levels and liveweight of grazing cattle when supplemented with *Leucaena leucocephala* or urea–molasses

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Abstract

Liveweight change and the concentration of a number of metabolites in rumen fluid, blood and faeces were monitored in cattle from weaning until they were 33 months old, grazing spear grass alone and when supplemented with either *Leucaena leucocephala*, urea–molasses, or both.

Levels of metabolites in unsupplemented cattle in winter reflected the poor protein content of spear grass. A low molar percentage of higher volatile fatty acids in rumen fluid (1.09 and 1.72% for weaners and two-year-old cattle, respectively) indicated a shortage of amino acids in the feed; and the low ammonia nitrogen concentrations (7 and 18 mg/L, respectively) would have limited bacterial protein production.

A true dietary protein supplement (leucaena) significantly ($P<0.05$) increased the molar percentage of higher volatile fatty acids in the winter to 1.60 and 2.57%, and in spring to 2.92 and 3.41% in yearlings and two-year-olds, respectively. Urea–molasses did not increase the molar percentage of the higher acids. Both supplements increased rumen concentrations of ammonia nitrogen in winter (weaners 105 and 72 mg/L, and two-year-olds 51 and 69 mg/L); but only leucaena increased concentrations in spring in both yearlings and two-year-olds (225 and 232 mg/L, respectively).

Weaners supplemented with leucaena gained liveweight in winter whereas weaners supplemented with urea–molasses lost weight although not as much as unsupplemented weaners. In spring, yearlings and two-year-olds supplemented with leucaena gained more than 0.9 kg/hd/day; but liveweight gain by urea–molasses supplemented cattle was not significantly ($P>0.05$) different from that of unsupplemented cattle.

We concluded that the better livewweight gains from leucaena were due to leucaena's superior amino acid profile.

INTRODUCTION

In the southern spear grass region of Queensland, winter liveweight loss in weaners was reduced and bare maintenance of liveweight in older cattle turned into a positive liveweight gain by feeding either non-protein nitrogen urea with molasses or a legume protein (*Leucaena leucocephala*) (Foster and Blight 1982).

We now describe the effects of these supplements on the levels of a number of metabolites monitored in rumen fluid, blood and faecal samples obtained from supplemented and unsupplemented cattle in the grazing experiment of Foster and Blight (1982). The seasonal variation in pasture quality is also described.

Significant differences in diet quality and metabolite values between the treatment groups are discussed in relation to intake of digestible organic matter, its fermentation in the rumen and the synthesis of bacterial protein, to partly explain the liveweight responses reported in Foster and Blight (1983, 1984).

MATERIALS AND METHODS

Cattle grazed spear grass (*Heteropogon contortus*) from 9 months of age to 33 months old during the period winter 1977 to autumn 1979. Cattle were allocated by stratified randomisation to four treatment groups:

NP, unsupplemented spear grass grazing (native pasture);

NP+UM, spear grass grazing+urea–molasses (10% urea, 78% molasses);

NP+L, spear grass grazing+leucaena; and

NP+L+UM, spear grass grazing+leucaena+urea–molasses.

Supplementation with urea–molasses ceased on 29 November, 1978. The leucaena had been established in 1969 and some green panic (*Panicum maximum* var *trichoglume* cv. Petrie) had volunteered below the leucaena shrubs.

In 1977 there were two replicates (paddocks) per treatment and four animals per paddock, giving a total of 32 yearlings and in 1978–79 three paddocks per treatment and two animals per paddock for a total of 24 two-year-olds. The sampling times and number of samples analysed to obtain the seasonal mean for all metabolites except faecal nitrogen are shown in Table 1.

Table 1. Number of samples of rumen fluid and blood taken on each collection day from individual animals in each treatment to obtain metabolite levels

Year	Season	Month	No. of collection days	No. of samples/ treatment	Total samples/ season
1977	Winter	August	2	8	16
	Spring				
	Before rain	October	3	8	
		November	3	8	48
	After rain	December	2	8	16
1978	Winter	June	1	6	
		July	1	6	12
	Spring	September	1	6	
		October	1	6	
		November	1	6	18
1979	Autumn	May	3	6	18

Strained samples of rumen fluid were obtained through rumen fistulae. Each sample was acidified with three or four drops of concentrated sulphuric acid and stored at −15°C. Total volatile fatty acid (VFA) concentrations and the molar percentages of the individual acids were estimated by gas chromatography (Erwin, *et al.* 1961) using iso-caproic acid as an internal standard with an automated gas chromatograph (GC HF Chromatron, Berlin). The concentration of ammonia nitrogen in the rumen fluid (rumen NH_3–N) was determined on a 5 mL sample of decanted fluid by steam distillation. The distillate was collected into 5% W/V boric acid, titrated with 0.05 N sulphuric acid and the endpoint detected using a pH meter.

Plasma urea nitrogen (PUN) was determined using the automated analyser technique based on the methods of Marsh *et al.* (1965). In 1977 rectal grab samples of faeces were taken from animals in the first replicate only and pooled. In 1978–79 faecal samples were taken from individual animals. Samples were dried at 80°C and analysed for faecal nitrogen (FN) by the procedure of Moir (1960).

Table 2. Metabolite values and daily liveweight change in winter, spring and autumn in unsupplemented and supplemented cattle grazing speargrass pasture

	ADG (kg/hd/day)	Total VFA (m mol/L)	Acetic	Propionic	Butyric	Higher acids	R-NH_3N (mg N/L)	PUN (mg N.100mL)	FN (%)
			(molar %)*						
Weaners, winter 1977 (20 Jun 1977 to 15 Sep 1977) 87 days									
NP	−0.230*c*†	59*b*	77*a*	14*d*	8	1.09*b*	7*c*	3*c*	1.0
NP+UM	−0.158*c*	69*b*	75*b*	16*c*	8	0.91*b*	105*a*	6*b*	
NP+L	0.099*b*	101*a*	71*c*	20*a*	8	1.60*a*	72*ab*	7*b*	2.2
NP+L+UM	0.194*a*	100*a*	73*c*	18*b*	8	1.55*a*	67*b*	8*a*	
LSD (*P*=0.05)	0.088	12	2	1	1	0.26	36	1	n.a.‡
Yearling cattle, Spring 1977									
Spring drought (15 Sep 1977 to 10 Nov 1977) 56 days									
NP	−0.172*b*	66*d*	78*a*	14*c*	7*b*	0.98*a*	4*b*	2*c*	1.0
NP+UM	−0.011*a*	72*c*	75*c*	16*a*	9*a*	0.80*b*	74*a*	5*a*	
NP+L	−0.127*b*	79*b*	77*b*	15*b*	8*b*	1.05*a*	14*b*	3*b*	1.1
NP+L+UM	−0.192*b*	84*a*	76*b*	15*b*	8*b*	1.10*a*	54*a*	6*a*	
LSD (*P*=0.05)	0.085	5	1	1	1	0.13	20	1	n.a.
After effective spring rain (10 Nov 1977 to 8 Dec 1977) 28 days									
NP	0.402*b*	76*b*	74*a*	15*c*	10	1.44*b*	55*b*	6*b*	1.7
NP+UM	0.429*b*	96*a*	74*a*	15*c*	10	1.35*b*	64*b*	6*b*	
NP+L	0.938*a*	103*a*	69*b*	19*a*	9	2.92*a*	225*a*	17*a*	3.0
NP+L+UM	1.000*a*	103*a*	70*b*	17*b*	10	3.02*a*	232*a*	17*a*	
LSD (*P*=0.05)	0.183	14	2	1	1	0.36	29	2	n.a.
Two-year-old cattle, winter 1978 (6 Jun 1978 to 29 Aug 1978) 84 days									
NP	0.018*c*	104*c*	75*a*	14*c*	10	1.72*b*	18*b*	9*c*	1.3*b*
NP+UM	0.242*b*	107*c*	74*b*	14*c*	11	1.49*b*	51*a*	14*b*	1.5*b*
NP+L	0.544*a*	136*a*	68*d*	19*a*	11	2.57*a*	69*a*	27*a*	2.1*a*
NP+L+UM	0.490*a*	116*b*	69*c*	17*b*	11	2.34*a*	62*a*	26*a*	2.1*a*
LSD (P=0.05)	0.141	6	2	1	1	0.41	20	5	0.3
Two-year-old cattle, spring 1978 (29 Aug 78 to 28 Nov 78) 91 days									
NP	0.623*b*	104*d*	74*a*	13*d*	10*b*	2.07*b*	57*d*	15*b*	1.6*b*
NP+UM	0.615*b*	121*c*	72*b*	15*c*	11*ab*	2.78*ab*	90*c*	20*b*	1.7*b*
NP+L	0.982*a*	153*a*	67*c*	18*a*	12*a*	3.41*a*	232*a*	41*a*	2.6*a*
NP+L+UM	0.874*a*	138*b*	68*c*	17*b*	12*a*	3.10*a*	178*b*	38*a*	2.6*a*
LSD (*P*=0.05)	0.109	12	2	1	1	0.87	26	5	0.2
Two-year-old cattle, autumn 1979 (17 Apr 1979 to 28 May 1979) 41 days									
NP	−0.053*b*	103*b*	77*a*	13*b*	8*b*	1.50*b*	13*b*	9*b*	1.3*b*
NP+L	0.488*a*	132*a*	71*b*	17*a*	10*a*	2.50*a*	108*a*	30*a*	2.1*a*
LSD (*P*=0.05)	0.389	8	2	1	1	0.19	17	3	0.1

* Expressed as a percentage of total VFA molar concentration.

†Lettering indicates a significant *F* test in the ANOVA for a set of means within columns and seasons. Means not having common letters are significantly different at *P*=0.05, within columns and season.

‡n.a.=not analysed.

The cell wall content (CWC) of herbage was estimated by sequential extraction with acid pepsin, ethanol and hot water by the procedure of Moir (1971). Cell wall was the residue from this extraction less ash and unextracted protein, estimated from its relationship with total protein as determined by a macro-Kjeldahl method. Undigested cell wall was

determined in the same way from residue, 0.6 g samples being digested *in-vitro* for 48 hours using the method of Tilley and Terry (1963). Digestible cell wall (DCW) was the difference between total cell wall (CWC) and undigested cell wall expressed as a percentage of the original organic matter. The apparent digestibility of forage the digestible organic matter (DOM), on offer was calculated using the equation of Moir *et al.* (1975):

$$DOM=89.3-CWC+DCW$$

Metabolisable energy (ME) was expressed as ME+0.16 DOM for leucaena leaf and stem; and ME=0.15 DOM for green panic and spear grass (MAFF 1975).

Cattle were weighed unfasted every 14 days in 1977 and every 28 days in 1978–79.

Winter 1977 was colder than average with 37 frosts and was also very dry. Only 8.6 mm of rain fell compared to an historical mean of 98.0mm. This dry period was prolonged through September and October and effective rain did not fall until 8 November. Winter temperatures in 1978 were average and a spring rainfall of 196 mm was above average (historical mean 168.0 mm). In May 1979, the minimum screen temperature was 10.1°C and one frost occurred. Only 4.0 mm rain fell which was well below the historical average for May (31.0 mm).

Analysis of variance was used to test for the effect of treatments on the levels of metabolites and average daily gain (ADG); error was estimated from variation amongst individual animals (Blight and Pepper 1982). The replicate×treatment interaction fixed effect was tested against error. Treatment means were compared using the protected LSD procedure at $P=0.05$. The protection in our LSD test involved the usual requirement of a significant F test for treatments in the analysis of variance, and the additional requirement that where the replicate×treatment interaction was significant, a qualitative judgement that it was not important.

RESULTS

Liveweight response

Unsupplemented weaners lost weight in winter and two-year-old cattle barely maintained weight (Table 2). Supplementation with urea–molasses tended to reduce winter weight loss in weaners ($P>0.05$), and only marginally increased their liveweight gain in spring ($P>0.05$). Weaners supplemented with leucaena made a small liveweight gain in winter and twice the liveweight gain of unsupplemented cattle in spring ($P<0.05$). In two-year-olds, supplementation with urea–molasses significantly ($P<0.05$) improved liveweight gain in winter, but had no significant ($P>0.05$) effect on liveweight gain in spring compared with unsupplemented animals. Leucaena supplement significantly ($P<0.05$) increased the liveweight gain of two-year-old cattle in both winter and spring.

Metabolite profile

Unsupplemented cattle

The metabolite profile (Table 2) of unsupplemented weaners in winter was characterised by low concentrations of rumen NH_3–N, PUN, total VFA and levels of faecal nitrogen that did not exceed 1.21% N. Following regrowth of green pasture in spring, the concentrations of rumen NH_3–N and PUN increased. There was also a 29% increase in total VFA with a decreased molar percentage of acetic acid and a proportional increase in the molar percentage of the other VFA. In two-year-old cattle, rumen NH_3–N concentration increased from 18 mg/L in winter to 57 mg/L in spring and PUN concentrations increased

from 9 mg/100 mL in winter to 15 mg/100 mL in spring. With the advent of spring there was no increase in the total VFA level in two-year-old cattle. There was, however, an increase in the molar percentage of the higher acids (iso-butyric, iso-valeric and valeric).

Supplemented cattle

Weaners and two-year-olds supplemented with either urea–molasses or leucaena in winter had significantly ($P<0.05$) higher concentrations of rumen NH_3–N and PUN than the unsupplemented animals. In spring after green pasture regrowth, except for a significantly ($P<0.05$) higher concentration of rumen NH_3–N in two-year-old cattle, the concentrations of rumen NH_3–N and PUN in unsupplemented cattle and cattle supplemented with urea–molasses were not significantly ($P>0.05$) different. In cattle supplemented with leucaena the concentrations of rumen NH_3–N and PUN were significantly ($P<0.05$) greater. Total VFA production was increased significantly ($P<0.05$) by both supplements in spring, but only by leucaena in winter. The molar percentage of the higher acids was significantly ($P<0.05$) higher in cattle supplemented with leucaena than in unsupplemented animals, except in the dry spring period (1977) when little leucaena leaf was available for grazing yearlings. There was a significant ($P<0.05$) simple correlation between the higher acids and concentrations of rumen NH_3–N in unsupplemented cattle and those supplemented with leucaena, but not for those supplemented with urea–molasses (Table 3). Urea–molasses supplement did not increase the molar percentage of the higher acids.

For cattle supplemented with both urea–molasses and leucaena, metabolite levels only reflected the individual treatment effects of each supplement but there were variable responses between replicates for this treatment group. There was no evidence to advocate the use of urea–molasses with leucaena to further increase final liveweight of cattle at 30 months of age (Foster and Blight 1982).

Table 3. Simple correlation coefficients between the molar percentages of the higher volatile fatty acids and rumen ammonia nitrogen in three treatment groups

	NP	NP+UM	NP+L
Iso-butyric	0.702*	−0.058	0.881*
Iso-valeric	0.360	0.072	0.894*
Valeric	0.683*	−0.102	0.802*

* Necessary value of coefficient for significance ($P=0.05$)=0.456.

Pasture quality, metabolite profile and liveweight response

In late autumn (17 April 1979 to 14 May 1979), before the first frost, 30-month-old cattle grazing spear grass gained 0.04 kg/hd/day while cattle given restricted access to leucaena gained 0.57 kg/hd/day. Differences between the two treatment groups in the quality of available herbage and the metabolite levels in the animals were determined from samples collected on 1 May 1979 (Table 4).

The liveweights of two-year-old cattle during the period of supplementation with leucaena show a cumulative advantage compared with unsupplemented cattle (Figure 1). An important variable in explaining seasonal variation in the liveweight response to leucaena was CWC ($r=-0.634$, $P<0.05$), which is inversely related to crude protein content (CP) of leucaena leaf (Figure 2). The treatment group receiving NP+L had higher levels of metabolites than the group receiving only NP (Figure 3): FN did not fall below the critical value of 1.2% FN (Foster and Blight 1984), rumen NH_3–N always exceeded 50 mg/L, and the total VFA concentration was greater than 132 m mol/L.

Table 4. Quality of herbage components grazed by two-year-old cattle prior to the first frost of winter and their associated metabolite profile* and liveweight gain when grazing either NP or NP+L

	NP	NP+L		
	Spear grass	L leaf	L stem	Green panic
Herbage quality				
Crude protein (%)	3.6	20.0	8.2	4.7
DOM (%)	37.3	68.8	27.5	38.9
ME (MJ/kg OM)	5.8	11.0	4.4	6.0
CWC (%)	81.0	27.0	66.0	78.0
	NP	NP+L		
	spear grass	Complete grazing diet		
Metabolite profile and liveweight gain				
Rumen NH_3–N (mg N/L)	14	146		
PUN (mg N/100 mL)	9	37		
Total VFA (m mol/L)	104	138		
Higher acids (molar %)	1.6	2.9		
FN (%)	1.3	2.5		
ADG (kg/hd/day)	0.04	0.57		

* Mean of six animals (see Foster 1982).

†Supplement period: 17 April 1979 to 14 May 1979.

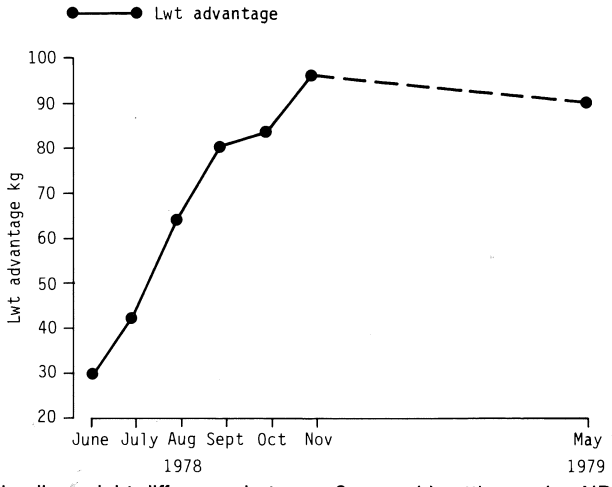

Figure 1. Cumulative liveweight difference between 2-year-old cattle grazing NP+L and cattle grazing NP only in winter, spring and autumn.

DISCUSSION

During winter spear grass pasture in south-eastern Queensland has a low content of sulphur and protein. We recorded 3.6% crude protein and 0.06% sulphur in late autumn (May 1979). The pasture also had a low metabolisable energy content of 5.8 MJ/kg organic matter (OM) (Foster and Blight 1983). Also we found that the concentrations of rumen NH_3–N in weaners and two-year-olds were low, indicating a shortage of soluble nitrogen in the rumen. The low molar percentage of the higher acids (weaners 1.09 and two-year-olds 1.72%) suggested a deficiency of amino acids since higher acids are the products of breakdown of amino acids in the rumen (El Shazly 1952*a*, 1952*b*). Consequently, rumen

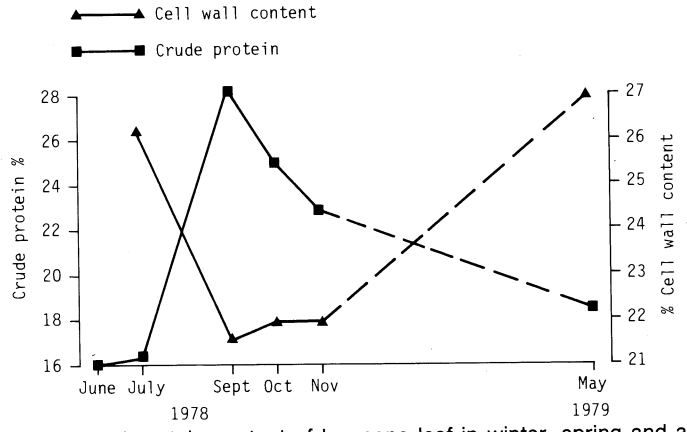

Figure 2. Cell wall and protein content of leucaena leaf in winter, spring and autumn.

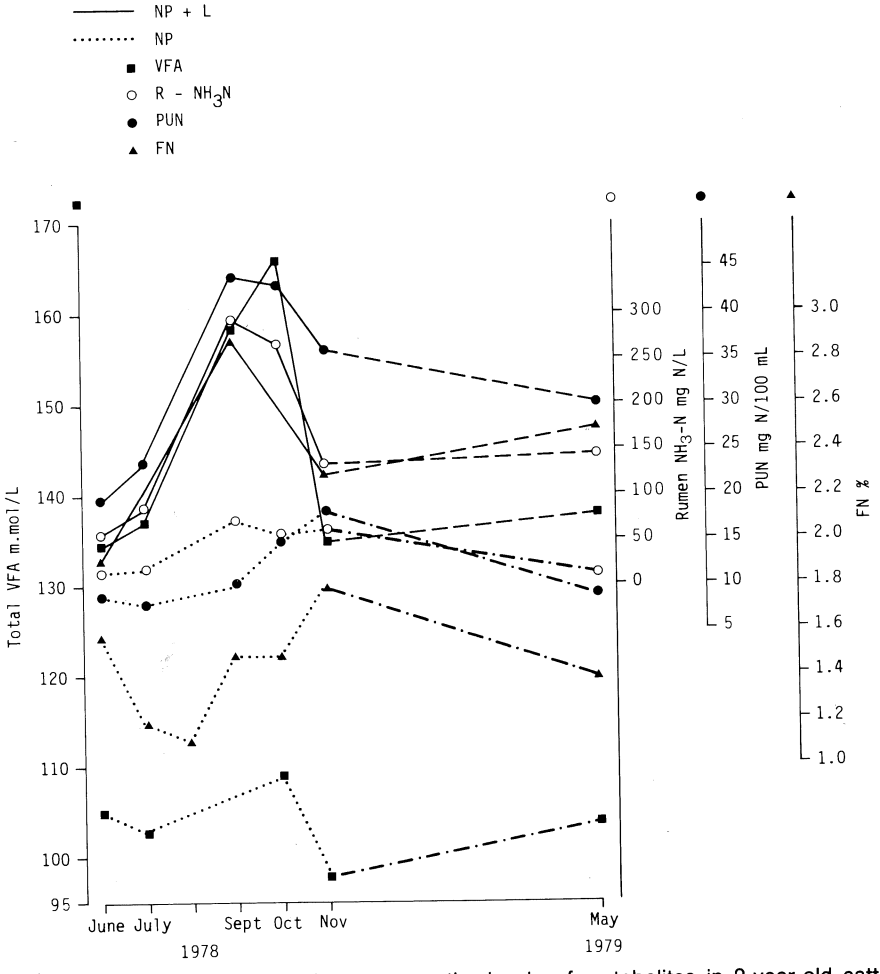

Figure 3. Effect of supplementation with leucaena on the levels of metabolites in 2-year-old cattle grazing spear grass in winter, spring and autumn.

fermentation and the synthesis of microbial protein would have been sub-optimal and although the deficiency would have been partly corrected by endogenous urea nitrogen, the quantity of protein leaving the rumen would not have been sufficient to maintain a desirable appetite and feed intake (Egan and Moir 1965). The high molar percentages of acetic acid (weaners 77% and two-year-olds 75%) were also indicative of low microbial protein synthesis (Hume 1970). In the spring, after regrowth of green pasture, the molar percentage of acetic acid decreased and there was a proportional increase in the molar percentages of the other VFA. During winter, spear grass barely maintained the liveweight of two-year-old cattle, while weaners lost weight because the pasture did not provide enough protein for their early growth (Orskov 1970).

Liveweight was not significantly improved by supplementation with urea–molasses except in yearlings during the spring drought (1977) and in two-year-old cattle during a short period in winter (1978) when the FN level in unsupplemented cattle fell below 1.2%. At these times the supplement only reduced weight loss. Ruminants have difficulty in synthesising sufficient glucose for their needs from amino acids and propionic precursors, particularly on low-protein diets such as the spear grass in our experiment during winter. In yearlings, the problem is more severe as the requirement for glucose synthesis competes with a high demand for amino acids for protein deposition. In this respect, the urea–molasses supplement would not contribute amino acid nitrogen to the body pool. The supplement did significantly change the fermentation pattern from acetate to propionate so that some glucogenic material resulted. The urea–molassses supplement also provided a readily available source of ammonia nitrogen in the rumen. The mean rumen NH_3–N concentration was increased in winter from 7 and 18 mg/L to 105 and 51 mg/L in weaners and two-year-olds, respectively. While these latter rumen NH_3–N levels may have been adequate (Satter and Slyter 1974), the lack of amino acids and peptides would have inhibited maximum microbial protein production (Maeng *et al.* 1976). This would explain the lack of correlation between rumen NH_3–N concentration and the molar percentage of the higher acids (Table 3) in the treatment group receiving NP+UM.

Leucaena browse contained 0.35% and 0.14% sulphur in leaf and stem respectively, (Foster and Blight 1983) and so supplemented the sulphur deficient spear grass (0.06% sulphur). It also provided a supplement of rumen digestible protein, undegradable digestible protein and metabolisable energy (11.68 and 5.26 MJ/kg OM in leaf and stem) (Foster and Blight 1983). In the NP+L treatment group rumen NH_3–N concentration exceeded 50 mg/L in winter and 220 mg/L in spring; amino acids and peptides would have been adequate. Relative to unsupplemented cattle the increased presence of higher acids at all times, and the significantly higher levels of VFA would have provided carbon skeletons required by cellulose-digesting bacteria for their growth (Bryant and Doestach 1955). For these reasons microbial protein synthesis would have been enhanced.

In steers, Aii and Stobbs (1980) found that only 16.1% of leucaena protein was lost in the first 12 hours of digestion in the rumen. In goats, Bamualim *et al.* (1984*a*) reported that 34% of fresh leucaena protein escaped rumen degradation, indicating that leucaena is a good source of undegradable digestible protein; amino acid absorption from the small intestine is increased, thus stimulating appetite and feed intake. In their pen trials with sheep Bamualim *et al.* (1984*b*) found that the addition of leucaena to a basal diet of spear grass and urea increased intake by 43%. In our grazing trial, the significantly higher levels of total VFA in the NP+L treatment group indicated that digestible organic matter intake increased by an amount similar to that reported by Kennedy and Siebert (1972) when lucerne was fed to cattle as a supplement to spear grass. Additional energy resulting from fermentation of the extra digestible feed intake would in turn increase microbial protein

production (Walker 1965) proportional to the ARC (1980) recommendation for microbial growth of 1 MJ ME (1.25 g N). All of these processes help to explain why cattle grazing spear grass and supplemented with leucaena had a liveweight advantage over unsupplemented cattle.

The exact amount and type of food intake by the grazing cattle was unknown and this limits our interpretation of the data. It was not possible to identify rumen digestible protein, undegradable digestible protein, energy or minerals as the primary factor in the liveweight response to leucaena. However, leucaena did provide a satisfactory amino acid spectrum (Foster 1982) while urea–molasses did not. Moreover, the winter liveweight response by weaner and two-year-old cattle was significantly greater in the NP+L treatment group than in the NP+UM treatment group, although rumen NH_3–N concentrations in these two groups were not significantly different. These observations suggest that amino acids may exert a major influence in increasing the energy intake and productivity of young cattle.

The quality of feed consumed and levels of metabolites in cattle of the NP and NP+L treatment groups differed (Table 4) to the extent that the supplemented cattle gained more weight. Our experiment was not designed to establish regression equations relating liveweight gain with diet quality or rumen and plasma metabolites. However, simple correlations do suggest that CWC (r=−0.634), PUN (r=0.638) and rumen NH_3–N (r=0.573) were the important variables in explaining seasonal variation in liveweight response by the cattle supplemented with leucaena between 21 and 32 months of age. The trend lines plotted in Figure 3 show that the metabolite levels were markedly increased when leucaena was fed, particularly in winter and early spring when microbial protein production in the rumen is critical to grazing cattle.

The study suggests that the inadequate energy, protein and sulphur content of spear grass in winter associated with poor feed intake led to low molar percentages of the higher acids and a deficiency of ammonia nitrogen in the rumen. Supplementation with urea–molasses reduced these deficiencies by specifically increasing the level of available ammonia nitrogen in the rumen for the growth of cellulose-digesting bacteria. However, supplementation during winter merely reduced weight loss in weaners while it increased the liveweight gain of two-year-old cattle. Supplementation with leucaena provided amino acid chains within the rumen, additional energy and sulphur and undegradable digestible protein directly to the small intestine. Except in drought, the liveweight response following restricted leucaena grazing greatly exceeded that resulting from supplementation with urea–molasses. Weaners gained weight in winter and in the other seasons both yearlings and two-year-old cattle receiving leucaena gained more liveweight than did cattle receiving urea–molasses.

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