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Energy and Protein Supplementation Can Improve Liveweight Gain of Steers Grazing Good Quality Tropical Pasture in the Wet Season

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Abstract: This experiment aimed at maximising wet season liveweight gain (LWG) of cattle grazing good quality tropical pasture. Twenty-five Brahman crossbred steers (203 ± 4.2 kg) were allocated into 5 treatments, namely control (Con; grazing only), grazing + molasses/urea mixture (MU) at 0.5% liveweight (LW) (5MU) or 1% W (10MU), and grazing + mixture of molasses/urea (55%), fish meal (25%) and whole cottonseed (WCS; 20%) at 0.5% W (5MWF) or 1% W (10MWF). Steers grazed fertilized pangola grass pasture (*Digitaria eriantha* cv. Steudal) for 84 days. Pasture DM availability was maintained at > 1.5 t/ha. The Mean green leaf yield was 1.9 t DM/ha, *in vitro* DM digestibility was 64%, and crude protein content was 15%. The LWG of Con steers was 960 g/d. Providing MU did not increase LWG, but inclusion of fishmeal and whole cottonseed markedly improved LWG above control by 34% and 39% for 5MWF and 10MWF, respectively. Levels of supplement had no affected on LWG. It is concluded that supplementing rumen fermentable energy and protein alone did not increase LWG of steers grazing good quality tropical grass pasture, but the inclusion of rumen bypass protein and energy in supplement significantly increased LWG, as a result of the higher bypass protein and energy intake.

Key words: Energy, protein, grazing, cattle, tropical pasture.

1. Introduction

To increase growth rates of cattle from tropical pastures, the nutrients available for tissue protein gain needs to be maximised. For which, one strategy is to increase the supply of fermentable organic matter (FOM) for a higher microbial protein supply [1]. However, there is only a limited amount of microbial crude protein (MCP) produced per kg digestible organic matter (DOM) and this is usually below tissue requirement for maximum growth rate of young animals [2]. Therefore, the feeding strategies should be directed toward maximising rumen fermentation and at the same time providing extra digestible protein to the intestines. Studies on grazing cattle [3, 4] demonstrated an improvement in LWG of grazing steers in the wet season when supplements of by-pass protein in the form of fishmeal or formaldehyde-treated casein were given. These results grant a strong ground to expect that supplementation of extra energy and/or protein to steers consuming good quality tropical grass pastures will have a positive effect on growth performance. The present experiment aimed at assessing the efficacy of supplements of molasses-urea mix with or without fish meal and whole cottonseed on growth rates of

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steers grazing a good quality pangola grass pasture in the wet season.

2. Materials and Methods

2.1 Experimental Site

The animal study was conducted at the University of Queensland Mount Cotton Research Farm, south east Queensland, from 1 January to 14 April (rainy season). The farm is situated at longitude 153°14′ E and latitude 27°53′ S. The local climate is subtropical with a predominantly summer rainfall incidence.

2.2 Experimental Design, Diets, and Treatments

A completely randomised design of 5 treatments with 5 replicates was employed in the study. The steers were assigned randomly into 5 groups of 5 on the basis of stratified unfasted liveweight. A 12 weeks data collection period was preceded by a period of 3 weeks adaptation to the pasture, supplement, and grazing management.

The supplement consisted of molasses, urea, WCS and fish meal. These ingredients were formulated into 2 different supplements and were given at 2 levels to the 2 treated groups. The combinations were: MU (97% molasses and 3% urea; w/w), and MWF [(55% molasses/urea (urea at 3% of molasses as above), 20% WCS and 25% fish meal)]. The proportion of ingredients in the ration was on the basis of weight as fed. The daily amounts of each of the supplements offered were 0.5% and 1% of liveweight (LW). Levels of supplement were kept similar to that of Bolam [5] to make a comparison in LWG response. The treatments of the present study were:

Con—Grazing only, no supplement
5MU—Grazing + MU (0.5% W)
10MU—Grazing + MU (1% W)
5MWF—Grazing + MWF (0.5% W)
10MWF—Grazing + MWF (1% W)

2.3 Pasture and Grazing Management

A 2.5 ha of pangola grass pasture, divided into two paddocks, were used as grazing sites. These paddocks were rotationally grazed. The paddocks were fertilized with 130 kg urea/ha at least 1 week prior to each grazing period and were occasionally irrigated as required using an automatic sprinkler system.

Total herbage DM on offer in each paddock was maintained above 1.9 t DM/ha. All steers grazed together and were brought into individual pens every morning at 06:00. The animals that received supplements were fed in individual pens. The control animals were kept in a large pen without supplement and were released back into the grazing site at the same time as those receiving supplements. The time the steers were kept in the pens varied from 4 to 7 h depending on the time required by the steers to consume the supplements.

2.4 Experimental Procedures

2.4.1 Pasture Mass and Quality

Herbage mass was monitored by taking 10-25 quadrates (40 cm × 40 cm) from each paddock one day before the steers entered and on the day they left the paddock. Herbage heights were also recorded in these quadrates by measuring from the ground to top of the leaf. The quadrates were cut close to the ground (approximately 3 cm above ground) using a hand shearer. All samples were oven-dried separately at 60 °C for 48 h to determine DM content. Four quadrates were randomly selected to determine herbage green leaf (GL), green stem (GS) and dead matter (D) proportion.

2.4.2 Supplement Intake and Sampling

Supplement intake was measured by weighing the amount offered and refused daily. The amount of supplement offered was adjusted every week based on the LW recorded at the end of the previous week. Daily samples of fish meal, and WCS were taken and bulked separately into containers in a refrigerator for the whole experimental period. At the end of the study, one sub-sample from each ingredient was taken, weighed, oven-dried, re-weighed, ground (WCS only)

and stored for chemical analyses. Daily sub-samples of molasses/urea were taken and bulked into a container in a refrigerator for a week. Two sub-samples of these ingredients were then taken at the end of the week. One sub-sample was stored in a freezer for N analysis, and another one was oven-dried for DM and organic matter (OM) determination.

2.4.3 Rumen Fluid

Two spot samples of the rumen fluid from all 25 steers were collected by means of stomach tube aspiration under vacuum. The first sampling was done in week 6 and the second was performed on the last day of the data collection period was completed. Sampling was done 3 to 6 h after the animals were fed the supplements. Collected rumen fluid was strained through 2 layers of stocking to separate coarse materials. About 40 mL of rumen fluid was acidified using 8 to 10 drops of concentrated H_2SO_4 to reduce the pH below 3. The acidified sample was then transferred into two 20 mL plastic containers and stored separately in a freezer prior to analysis for VFAs and NH_3-N.

2.4.4 Plasma Urea

Two blood samples were taken at the same time as that of the rumen fluid. A 20 mL blood sample was collected from the jugular vein by syringe. The blood was transferred into two 10 mL heparinized tubes, swirled gently and temporarily buried in crushed ice. After collecting all blood samples, the tubes were centrifuged at 3,000 rpm for 15 min. The plasma was then pipetted into two 5 mL plastic tubes and frozen prior to determination of urea concentration.

2.4.5 Liveweight Gain

Fasted liveweight was obtained at the beginning and end of the experiment. In addition, animals were weighed twice a week unfasted in the morning (06:00) before the supplements were offered. Daily LWG was estimated from slope of the regression of the liveweight against time for each steer. The regression was used to minimise the influence of changes in gut fill on liveweight.

2.5 Analytical Procedures

2.5.1 Nutrients

Procedures for DM determination in pasture samples, molasses, fish meal, WCS, and faeces were done by drying samples at 60 °C until constant weight was achieved [6]. Ash content was determined by combusting approximately 2 g oven-dried ground sample in a furnace at 550 °C for 4 h. Ash free neutral detergent fibre (NDF) content of the WCS, fishmeal, and herbage fractions was determined by the method of Van Soest and Wine [7] using a cellulose and fiber determination extractor (Dosi-Fiber 6 units code 4000623, J.P. Selecta, Abrera, Spain). One gram extracted NDF was then burned in furnace at 550 °C for 4 hours for ash determination. Total N content of herbage fractions (GL, GS, and D), molasses/urea mix, fishmeal, WCS, and faeces was determined according to Sweeney [8] using an automated organic nitrogen determinator (LECO FP-42, St. Joseph, MI, USA). Lipid content of fish meal and WCS was determined by the ether extract (EE) method [6] using a solvent extraction unit (Soxtec HT6, Tecator, Sweden).

2.5.2 Concentration of Rumen Volatile Fatty Acids, Ammonia Nitrogen, and Plasma Metabolites

Total concentration and molar proportions of VFAs in the rumen fluid samples were quantified according to Cottyn and Boucque [9] using gas liquid chromatography technique (GLC; Hewlett Packard 8530A with a Hewlett Packard 18850A gas chromatography terminal, and HP3396A integrator; Packard Instruments Corporation, New Jersey, USA). Concentration of rumen NH_3-N was determined by distillation method [6] using a Büchi 321 distillation unit and an automatic titrator (Büchi Scientific Apparatus Flawil, Switzerland). Plasma glucose concentration was determined by an enzymatic method (peridochrome® glucose) using method of Tiffany et al. [10]. A spectrophotometer (2000 UV Visible Spectrophotometer; Beijing scientific instruments & materials co) was used to measure absorbance at the wavelength of 340 nm and a

temperature of 27 °C.

2.6 Statistical Methods

Data were subjected to an analysis of variance (ANOVA) according to a 5 × 5 completely randomised design [11] with the aid of MicroQuasp version 3.02 [12] to compare the effects of the treatments. Differences in the treatment effects between groups were declared significant at $P < 0.05$.

3. Results

3.1 Seasonal Condition

Weather data were obtained from University of Queensland Mount Cotton Research Farm. A total of 451 mm of rain occurred during the study in January and April. The average minimum and maximum temperature was 21 °C and 27 °C respectively.

3.2 Herbage Mass and Quality

Four fertilized pangola grass paddocks with a total grazing area of 2.4 ha were used as grazing site. Results of the pasture measurements are presented in Table 1. Total herbage DM on offer for the 4 paddocks was maintained above 2 t DM/ha except paddock B1 where total DM was only 1.6 t DM/ha when animals left the paddock after the first grazing period. There was a marked decline in the yield of GL on offer from 1.9 to 0.7 t DM/ha between the animals entered and left the experimental paddocks, averaging over all paddocks. The proportion of the GL in the total DM declined, on average, from 430 g/kg DM to 233 g/kg DM before and after grazing, respectively whilst the proportion of GS and D components increased gradually toward the end of each grazing period in each of the paddocks. Pasture height varied from 12 to 30 cm over the study period.

3.3 Herbage Chemical Composition

The chemical composition and IVDMD of the GL, GS, and D components from each paddock are shown in the Table 2. Overall mean values of the paddocks before and after grazing indicated that CP (158 g CP/kg DM) and IVDMD (59%) content of GL were higher than GS (81 g CP/kg DM and 52% for CP and IVDMD, respectively) and D (92 g CP/kg DM and 34% for CP and IVDMD, respectively). NDF content was lower for GL than GS and D both before and after grazing across paddocks.

Table 1 Mean herbage height, mass, and fractions in the experimental paddocks as steers entered and left the paddocks.

Grazing status	Herbage height	Herbage mass	Green leaf	Green stem	Dead matter
	cm	kg DM/ha	-------- g/kg total DM--------		
Entered	24	4769	430	294	276
Left	16	3630	233	345	422

Table 2 Chemical composition of herbage and the supplements fed to steers grazing pangola pasture in the wet season.

Ingredient	DM	OM	CP	EE	NDF	IVDMD
		g/kg DM				%
Supplements						
Whole cottonseed	927	961	203	172	484	-
Fish meal	898	817	703	94	-	-
Molasses	676	836	63	-	-	-
Molasses/urea mix	664	808	161	-	-	-
Herbage						
Steers entered the paddocks						
Green leaf		916	174	-	665	64
Green stem		939	90	-	740	55
Dead matter		934	94	-	735	37
Steers left the paddocks						
Green leaf		917	142	-	709	55
Green stem		943	71	-	747	50
Dead matter		945	89	-	755	31

3.4 Composition of the Supplements

The composition of WCS, fish meal and the molasses/urea mix is presented in Table 2. Twelve batches of molasses were used throughout the experimental period.

3.5 Animal Performance

3.5.1 Liveweight Response to Supplementation

The MU supplement had no effect on LWG relative

to the control (Table 3). The MWF groups gained up to 39% more weight than those of control and MU groups. Increasing intake of MWF supplement from 0.38% to 0.76% liveweight (W) did not increase LWG significantly ($P > 0.05$).

3.5.2 Supplement Intake

The intake of supplements (as fed) of 5MWF and 10MWF almost reached the intended intake but animals on treatment 10MU consumed only 80% of intended intake of supplement. Total time spent consuming the supplements was usually longer (up to 7 h) for the first 3 to 4 days after the steers started grazing a new paddock. Total intake of DM, OM, CP, and EE of supplement are presented in Table 3. The results showed that MWF groups consumed more CP than MU groups at the same level of supplementation. Inclusion of WCS and fish meal in the MWF diet resulted in an appreciable amount of lipid consumed by the steers. There was no significant difference ($P > 0.05$) in total faecal CP content between treatments (Table 3).

3.5.3 Ammonia Nitrogen and Volatile Fatty Acid Concentrations in the Rumen Fluid

Table 4 shows the NH_3-N and VFA concentration in the rumen fluid. There were no period effects for samples taken in week 6 or week 11 so the mean values are presented. Rumen ammonia-N concentrations were marginally elevated ($P < 0.05$) by supplementation but only treatment 10MWF was

Table 3 Mean nutrient intake from supplement and liveweight gain (LWG) of the steers grazing pangola pasture alone (Con) or supplemented with molasses/urea at an intake of 0.5% liveweight (5MU) or 1% liveweight (10MU) or a mixture of molasses/urea, whole cottonseed and fish meal at an intake of 0.5% liveweight (5MWF) or 1% liveweight (10MWF).

Parameter	Treatment					SEM	Sig
	Con	5MU	10MU	5MWF	10MWF		
Supplement intake							
Dry matter (g/d)	-	798	1,244	989	2,045		ns
Dry matter (%W)	-	0.32	0.52	0.38	0.76	-	ns
Organic matter g/d)	-	646	1,015	841	1,744	-	-
Crude protein (g/d)	-	129	202	334	692	-	-
Ether extract (g/d)	-	-	-	58	120	-	-
LWG (g/d)	960[a]	970[a]	914[a]	1,288[b]	1,335[b]	52.7	**

Different alphabetical superscripts within rows denote a significant difference (**$P < 0.01$; ns, not significant)

Table 4 The concentration of rumen ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFAs) in rumen fluid, and plasma urea of steers grazing pangola pasture alone (Con) or supplemented with molasses/urea at an intake of 0.5% liveweight (5MU) or 1% liveweight (10MU) or a mixture of molasses/urea, whole cottonseed and fishmeal at an intake of 0.5% liveweight (5MWF) or 1% liveweight (10MWF).

Parameter	Treatment					SEM	Sig
	Con	5MU	10MU	5MWF	10MWF		
Rumen fluid							
NH_3-Nitrogen (?) (mg/L)	199[a]	231[a]	240[a]	237[a]	279[b]	14.7	*
VFA (mmol/L)	36.6	48.4	42.3	46.6	40.6	3.76	ns
Molar proportion (%) of VFA							
Acetic acid	68.6	64.3	65.9	70.7	67.7	1.92	ns
Propionic acid	17.1	19.9	17.4	16.9	17.5	1.31	ns
Butyric acid	11.5[a]	14.3[b]	14.5[b]	10.4[a]	12.3[ab]	0.87	*
Iso-butyric acid	1.2[a]	0.6[b]	0.6[b]	1.0[a]	0.9[ab]	0.13	*
Valeric acid	0.6	0.6	1.2	0.4	1.0	0.27	ns
Iso-valeric acid	0.9[a]	0.2[b]	0.3[bd]	0.6[c]	0.5[cd]	0.09	**
Plasma metabolites (mg/dL)							
Urea	36[a]	56[b]	67[bc]	70[c]	111[d]	3.9	**
Glucose	84	74	70	83	83	2.1	ns

Different alphabetical superscripts within a row denote a significant difference (*, $P < 0.05$; **, $P < 0.01$; ns, not significant $P > 0.05$).

significantly different from control. Total VFA concentration in the rumen was not affected ($P > 0.05$) by supplementation. The ratio of C_2 equivalent (acetate acid + 2 × butyrate acid) to propionic acid was in a range of 4.7 to 5.4 and not significantly ($P > 0.05$) affected by supplementation. However, supplementation with MU alone increased the molar proportion of butyric acid.

3.5.4 Plasma Urea and Glucose Concentrations

Plasma urea-N concentration was affected by type and level of supplements (Table 4). Increasing level of MWF significantly increased plasma urea-N concentration ($P < 0.01$) but there was no such trend with MU diets. Concentration of plasma glucose was not significantly affected ($P > 0.05$) by any supplementation.

4. Discussion

4.1 Liveweight Gain

The LWG found in the present study (Table 3) is similar with the conclusion drawn by t'Manetje [13] that LWG of cattle grazing tropical pastures without supplement was below 1 kg/d. The supplements were designed to test whether animals would respond to simple supplementation of the rumen or a combination of rumen and bypass protein and lipid supplements.

No response to molasses-urea mix in the current experiment is in agreement with that of Winks et al. [14, 15] who found no response in LWG to molasses-urea supplementation in cattle grazing tropical pasture in summer. A similar result was also observed for pangola pastures [16]. In contrast, Tierney et al. [17] reported an improvement in LWG by up to 83% above control for steers grazing fertilized pangola grass pasture and supplemented with unrestricted molasses in January/May. However, in their experiment the positive response to molasses might be confounded by low pasture availability (< 1 t DM/ha) toward the end of the study. In two other experiments, they [17] did not observe any effect of molasses supplementation. These findings suggested that molasses/urea supplementation was not an appropriate choice for cattle grazing high quality and high DM allowance tropical pastures, and that the extra MCP, if it occurred, was too small to result in a significant increase in LWG.

In contrast to MU, incorporation of WCS and fishmeal into molasses/urea (MWF) markedly increased LWG by up to 34% above unsupplemented steers, at 0.5% W inclusion, but there was no further significant increase at the higher level of MWF intake. It is not possible to determine the extent of substitution, if any in this situation, as intake was not measured and is difficult to do in the field. However, an improvement in the LWG means an overall increase in the nutrient intake even if substitution occurred. A positive response to fishmeal inclusion into molasses/urea mix was also reported by Cuban researchers [18] that addition of 242 g fishmeal into 2.8 kg molasses (2.6% urea in molasses) increased LWG of Brahman bulls fed freshly-harvested napier grass by 580 g/d compared to those receiving only molasses/urea. Other studies [3, 4] demonstrated that supplementation with bypass protein of fishmeal or formaldehyde-treated casein to steers grazing high quality tropical pastures increased LWG up to 300 g/d above that of the controls. Similarly, Mullik and Permana [19] demonstrated that providing legume forages as supplement to heifers grazing native pasture in the wet season resulted in an increase in LWG by up to 205% above unsupplemented group. Improvement in the LWG by fishmeal and WCS supplementation in the present study is expected since these two feeds supply additional protein and lipid that might be used more efficiently because of their bypass characteristics even if some substitution occurred.

Increases in LWG and N retention have been observed in response to providing post-ruminally, protein or amino acids to lambs consuming fresh pasture [20]. These results support the conclusion of

Klopfenstein [2] that protein supply to the intestine is the most limiting nutrient for maximum growth rate of cattle at pasture where intake is set by some diet characteristic. A theoretical calculation by Poppi and McLennan [1] indicated that extra protein of 150 g/d delivered to the small intestines would result in an extra LWG of 300 g/d.

4.2 Pasture Intake

Pasture allowance is often the most limiting factor for intake [21]. However, as discussed earlier for tropical grasses, GL allowance has the major effect on pasture intake. Van Dyne et al. [22] showed that grazing animals tend to select more leaf. Although diet selection was not measured in this study, there was a substantial decline in the proportion of GL of the total DM from 45% before grazing to 29% after grazing which suggested that steers selected more GL than the other two fractions (GS and D). A marked reduction in the proportion of GL from 33 to 8% in the sward of setaria grass (*Setaria sphacelata*, cv. Kuzungula) during 15 d grazing has also been documented [23]. Other studies [22, 24, 25] consistently showed a greater proportion of GL in the diet selected by grazing ruminants compared with the average of the vegetation to which they had access. Because of the selection capability, the quality of diet selected by the animals is usually higher than the average measured in the paddock [24]. The reason for this behaviour pattern is probably associated with the higher CP and lower structural carbohydrate concentrations in leaf than stem which also results in lower retention time in the rumen and higher intake [26].

4.3 The Effect of Supplementation on Rumen Fermentation and Blood Metabolites

Ammonia is utilized mainly by celullolytic bacteria for protein synthesis so low rumen NH_3-N concentration may result in low fibre digestion [27]. Minimum NH_3-N concentration has long been suggested to be 50 mg NH_3-N/L rumen fluid [24] but latter studies [26] suggested a much higher figure (up to 200 mg NH_3/L) which depended upon dietary protein and fermentable carbohydrates. The concentration of NH_3-N in the rumen fluid of the control and supplemented steers in the current study was very high. For the control group the NH_3-N concentration (199 mg NH_3-N/L) was almost 4 times the minimum requirement for optimum microbial synthesis (50 NH_3-N/L) [28]. This high rumen NH_3-N concentration can be explained by the high CP of pangola grass pasture used here (Table 4). Given the higher ammonia levels in the cattle on pangola diets, one would have expected an addition of molasses to enhance the conversion of ammonia into MCP, hence lowering rumen ammonia concentrations and increasing MCP. However, this effect was unlikely as urea was also added into the supplement to ensure adequate rumen degradable nitrogen (RDN) for supplement and would also contribute to the rumen NH_3-N pool.

Inclusion of fishmeal and WCS into MU provided extra protein into the rumen, some of which would have been degraded by microbes thus elevating rumen NH_3-N concentration above the control (199 vs 237 and 279 mg NH_3-N/L for the control, 5MWF and 10MWF, respectively). The extra total CP intake above MU groups was 205 and 490 g CP/d for 5MWF and 10MWF, respectively. In general, high NH_3-N would not be expected to be limiting for either rumen microbes or intake [28, 29] in the present study.

A significant increase in proportion of butyric acid (Table 4) associated with MU supplementation in the present study is consistent with finding from molasses-fed cattle in other study [30]. Higher butyric and acetic acid and low propionic acid indicates that the metabolic fate of molasses fermentation is mainly acetyl-CoA, and little is converted to oxaloacetate or lactate which are the precursors for propionic acid. This may be associated with the nutritional properties of the supplements which were not glucogenic in

nature. With respect to gluconeogenesis, these results suggested that the proportion of glucogenic VFA derived from the supplement did not significantly alter glucogenic capability as was reflected by a lack of a treatment effect on plasma glucose concentration (Table 4).

5. Conclusion

The main finding of this experiment is that growth rate of steers grazing fertilized pangola grass pasture in the wet season can be increased by about 300 g/d by supplementation of a mixture of molasses/urea, WCS and fishmeal but not with MU alone.

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