REGULAR ARTICLES



Effects of protein-energetic supplementation frequency on growth performance and nutritional characteristics of grazing beef cattle

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Abstract Two experiments were conducted to evaluate reduced supplementation frequencies for grazing beef cattle in rainy season. In experiment 1, evaluating the nutritional parameters, four rumen-cannulated Nellore bulls (BW = 410 kg) were used. In experiment 2, evaluating animal performance, 48 Nellore bulls (BW = 358 kg) were used. The treatments were as follows: mineral supplement (MS) alone and MS plus protein-energy supplement provided $3\times$, $5\times$ and $7\times$ /week. Supplementation frequency did not affect (P > 0.05) intake and digestibility. Average daily gain was greater (P < 0.001) to supplementation compared with MS. The supplementation $5 \times$ /week resulted in greater weight gain per hectare (9.24) and higher economic returns during the study period (1.64%) compared to other supplementations. Supplementation 5×/week increased animal performance and positively influenced economic returns.

Keywords Bulls · Controlled supplementation · Forage · Profitability

Introduction

In the rainy season, tropical forages have sufficient levels of crude protein, but a high concentration of insoluble nitrogen in neutral detergent, which is slowly and incompletely degraded

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² Faculdade de Medicina Veterinária, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil in the rumen, resulting in nitrogen deficiency for rumen microorganisms (Detmann et al. 2014).

One of the alternatives that have been increasingly used in production systems is the reduction in the frequency of the supply of supplements to animals kept in pastures, which reduces supplementation costs and does not compromise animal performance (Cappellozza et al. 2015). Furthermore, reduced supplementation frequency can improve the logistics of supplement supply and reduce labour and equipment costs (Moraes et al. 2017.)

Supplements may be given to cattle at intervals greater than 1 day without impairing the benefits of supplementation, which continue after the ingestion of a concentrate. This may be due to the metabolic adaptation of the animals as they become more efficient, especially in terms of nitrogen (N) use efficiency through increased N recycling, maintaining N levels for microbial use between supplementation intervals (Moraes et al. 2010).

Few studies have investigated a reduction in the frequency of supplementation in the rainy season. We hypothesised that such a reduction can reduce production costs without affecting the performance and the nutritional characteristics of Nellore bulls kept in tropical pastures.

The objective of this study was to evaluate the effects of supplementation frequency on performance, financial return and nutritional characteristics of Nellore bulls grazing in tropical pastures in Brazil during the rainy season.

Material and methods

Description of the study site

The experiments were performed at the Beef Cattle Rearing Sector of Universidade Federal de Mato Grosso (UFMT),

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Brazil, during the rainy season. Animal care and handling procedures followed the guidelines of the UFMT.

Experimental design and treatments

Experiment 1 was developed to evaluate the nutritional parameters of beef cattle in pastures. Four rumen-cannulated Nellore bulls (BW = 410 kg) were randomly distributed into a 4×4 Latin square design over four experimental periods of 21 days each, with 13 days for adaptation to the supplement and 8 days for sample collection. Prior to the experiment, the animals were weighed, labelled and allocated into four paddocks of 0.24 ha each of Urochloa brizantha cv. Marandu; the paddocks were equipped with waterers and individual feed bunks.

In experiment 2, animal performance was evaluated using 48 Nellore bulls (BW = 358 kg). The experiment consisted of a completely randomised design with 16 paddocks (0.82 ha) of Marandu (experimental unit) and was conducted over a period of 84 days.

Treatments (Table 1) were based on mineral supplement ad libitum and protein-energy supplements provided each day (7×), 5×/week (Monday to Friday) and 3×/week (Monday, Wednesday and Friday) at 10 a.m., supplied at amounts (0.4% BW) of 1.5, 2.1 and 3.5 kg/animal, respectively. All treatments received the same amounts of supplement weekly

Composition of supplement (g/kg) and chemical composition Table 1 of supplements and forage (% DM)

	Supp	lements	Pasture ^b		
	MS ^a	Standard	Exp. 1	Exp. 2	
Ingredients					
Corn	_	300	-	_	
Soybean meal	-	300	-	_	
Whole cottonseed	_	300	_	_	
Urea-AS (9:1) ^c	_	30	_	_	
Mineral supplement ^d	100	70	-	_	
Chemical composition					
Dry matter	_	923	256	241	
Crude protein	_	303	81	91	
Neutral detergent fibre (NDF)	_	260	681	647	
NDF corrected for ash and protein	_	227	636	608	
Indigestible neutral detergent fibre	_	91	161	130	
Neutral detergent insoluble nitrogen ^e	_	22	9	11	

^a Mineral supplement

^b Samples obtained by simulated grazing

^c Urea-ammonium sulphate

^dMS-guaranteed levels (kg):120 g calcium, 30 g phosphorus, 25 g sulphur, 80 g sodium, 330 mg copper, 950 mg manganese, 1220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium and 300 mg fluorine e % of the total N

(10.5 kg/animal). Supplements were formulated to meet the nutritional requirements (30% crude protein (CP)) of Nellore bulls (BW of 395 kg) (Valadares Filho et al. 2016).

The animals for experiment 2 were weighed at the beginning and the end of the experiment after a fasting period of 16 h. After initial weighing, the animals were randomly distributed to 16 paddocks (three animals/paddock; four paddocks/treatment) and received the supplements.

Handling, measurements and samples

Two forage samples were collected at average height (50 points) every 21 days (Exp. 1) and 28 days (Exp. 2) by clipping all forage within a 0.25-m² frame in each paddock. Paddocks had an average forage mass of 5.7 (Exp. 1) and 2.0 (Exp. 2) ton/ha of dry matter (DM). Forage samples for the determination of the chemical composition were collected by manual simulation of grazing.

Faecal DM excretion was determined by using titanium dioxide (15 g/animal/day) as an external marker, which was introduced into the rumen once daily at 09 a.m. for 8 days. Subsequently, faecal samples were collected according to Smith and Reid (1955). Forage and faecal samples were airdried for 72 h (55 °C) and ground in a knife mill to pass through a 1-mm screen sieve.

To estimate urine and urea daily excretion, spot urine samples were collected 4 h after supplementation for three days (Saturday, Sunday and Monday). Urine samples (10 mL) were diluted with 40 mL of H₂SO₄ (0.036 N). To estimate plasma urea, blood samples were collected from the jugular vein, centrifuged $(4000 \times g \text{ for } 15 \text{ min})$ for serum extraction and stored at $-20 \text{ }^{\circ}\text{C}$.

Ruminal fluid was collected prior to supplementation and 4 h afterward on 3 days (Tuesday—day 1, Wednesday—day 2, Thursday-day 3). The pH value was measured using a digital pH meter. Subsequently, 1 mL of H₂SO₄ (1:1) was added to the ruminal fluid, and the mixture was stored at - 20 °C for the analysis of ammonia-N concentration.

Economic evaluation was carried out in view of the return on invested capital, dividing by the total investment profit margin involved in the supplementation process, considering a carcass yield of 52%. Revenue was obtained by multiplying carcass gain equivalent to the value in the region (US\$40.87); costs were obtained from the total cost of the pasture (15% of average value at sign during the study period + costs: operating, health, labour, work (US\$0.041/day)), cost of the supplement (kilogram value) and cost of the animals, with updates from the beginning to the end of the experiment in relation to interest rates (0.6%/month).

Chemical composition analysis

Forage, faeces and supplement ingredient samples were analysed for DM and CP according to the AOAC (2000).

Neutral detergent fibre (NDF), residual nitrogen compounds and indigestible neutral detergent fibre (NDFi) were analysed as described by Mertens (2002), Licitra et al. (1996) and Valente et al. (2011), respectively.

The DM intake was estimated using the NDFi as an internal marker and calculated by the equation proposed by Detmann et al. (2001).

Analysis of creatinine and urea levels in urine was according to Barbosa et al. (2011). Daily excretion of urinary urea N was according to Rennó et al. (2000).

Statistical analysis

The data were analysed using the mixed procedure of SAS (SAS9.3). The variables (Exp. 1) were analysed as a 4×4 Latin square design. The model statement used for intake and digestibility included treatment as fixed effect and animal and period as random effects. For the analysis of the nitrogen balance, efficiency microbial synthesis, ruminal pH and ruminal ammonia nitrogen, the model contained treatment, day, time and all interactions as fixed effects and animal and period as random effects. For ruminal pH and ruminal ammonia concentration, the model also included time within day as specified term for repeated measure. 'Unstructured' was determined to be the covariance structure based on the Bayesian information criterion. The Fisher DMS test was carried out for ruminal pH and ruminal ammonia nitrogen at the evaluation times.

The model statement used for average daily gain (ADG) (Exp. 2) included fixed effect of treatment, using the average of a paddock as experimental unit; initial BW was used as covariate. All results are reported as least square means. For the two experiments, contrast was used to compare mineral supplement versus supplementation frequency. Linear and quadratic effects of the increase in the supplementation frequency also were tested. Effects were declared significant at P < 0.05.

Results

Bulls that received protein-energy supplements had a greater (P < 0.05) intake of DM and CP than MS animals. A reduction of the supplementation did not affect (P > 0.05) nutrient intake. Digestibility of CP and NDF was greater (P < 0.05) for supplemented bulls compared with that of MS, but did not differ significantly (P > 0.05) between frequencies (Table 2).

A treatment × day interaction was observed for ruminal pH (P < 0.001); ruminal ammonia nitrogen (RAN) concentration showed a treatment × day × time interaction (P = 0.0063).

The MS animals presented greater (P < 0.001) ruminal pH values than those supplemented on day 3 (Table 3). On day 2, ruminal pH decreased linearly (P = 0.01) with increasing supplementation frequency. Animals of treatment 5× and 7× on day 1 and treatments 3×, 5× and 7× at days 2 and 3 had greater (P < 0.05) RAN at time 4 compared to time 0 (before supplementation).

At all days, no treatment effects were detected (P > 0.05) for RAN (time 0), but generally, RAN (time 4) was greater with supplementation compared with MS. On days 1 and 2, RAN responded quadratically (P < 0.05) as the supplementation frequency increased at time 4; however, on day 2, no treatment effects were detected at time 4. In general, RAN was greater at time 4 compared with time 0, except for MS and 3× (on day 1), where no differences were detected.

Table 2Intake and digestibilityof nutrients by beef cattlesupplemented with mineralsupplement or concentrate (Exp.1)

	Supplements				SEM	Contrasts		
	MS ^a	$3 \times^{b}$	$5 \times^{b}$	7× ^b		$MS \times Freq$	L	Q
Intake in DM (kg/day)								
Dry matter	10.2	11.0	12.2	12.0	1.12	0.048	0.242	0.320
Forage	10.1	9.6	10.8	10.6	1.12	0.716	0.244	0.321
Supplement	0.1	1.4	1.4	1.4	0.01	_	_	_
Crude protein	0.8	1.2	1.3	1.3	0.09	< 0.001	0.265	0.281
Neutral detergent fibre	6.9	6.9	7.7	7.6	0.75	0.245	0.252	0.326
Digestibility (%)								
Dry matter	61.1	60.4	62.9	62.4	2.75	0.560	0.286	0.355
Crude protein	50.4	59.4	58.8	62.4	3.52	0.002	0.249	0.338
Neutral detergent fibre	65.1	61.6	63.6	62.7	2.51	0.009	0.211	0.083

 $MS \times Freq$, MS vs. supplemented; L, linear effect within frequency of supplementation; Q, quadratic effect within frequency of supplementation

^a Mineral supplement

^b Frequency of supplementation (7×, 5× and 3×/week)

Table 3Ammonia nitrogenvalues and pH of Nellore bullssupplemented with mineralsupplement or concentrate (Exp.1)

Collection days ^a Ti	Time	Suppl	ements			SEM	Contrasts			
		MS	3×	5×	7×		$MS \times Freq$	L	Q	
Ruminal pH										
Day 1	-	6.5	6.4	6.6	6.6	0.18	0.800	0.113	0.470	
Day 2	-	6.6	6.7	6.5	6.4	0.18	0.512	0.016	0.370	
Day 3	_	6.8	6.5	6.5	6.5	0.17	< 0.001	0.948	0.813	
Ruminal ammonia	nitrogen	(mg/dL)	1							
Day 1	0	7.4a	10.5a	7.6a	5.5a	2.54	0.857	0.103	0.878	
	4	7.1a	9.9a	21.2b	16.5b	3.55	0.001	0.032	0.003	
Day 2	0	4.4a	5.2a	7.7a	6.5a	1.36	0.503	0.658	0.510	
	4	7.9a	14.1b	15.0b	11.4b	2.44	0.058	0.473	0.442	
Day 3	0	5.7a	9.0a	6.3a	4.3a	1.63	0.793	0.200	0.921	
	4	6.9a	6.8b	22.5b	12.6b	1.92	0.005	0.060	< 0.001	

Means followed by different letters in the same column within a day are statistically different from each other by the Fisher DMS test at 5%

^a Day 1 = Tuesday ($5 \times$ and $7 \times$ were supplemented), day 2 = Wednesday (all animals were supplemented), day 3 = Thursday ($5 \times$ and $7 \times$ were supplemented)

There were no treatment × day interactions for variables related with nitrogen use (P > 0.05), but we found treatment effects (P < 0.05; Table 4). Animals supplemented had greater (P < 0.05) nitrogen intake (NI), excretion of nitrogen in urine (ENU), urine urea nitrogen (UUN), excretion of nitrogen in faeces (ENF), serum urea nitrogen (SUN) and N balance (NB) than those on MS. Furthermore, increasing the frequency of supplementation had a quadratic effect (P < 0.05) on NI and ENF. Absorbed N and NB increased linearly (P < 0.05) with an increasing supplementation frequency. Supplementation frequency had no effect (P > 0.05) on ENU, UUN and SUN.

There were no significant differences (P > 0.05) between the treatments in terms of absorption (203 mmol/day) and total excretion (194 mmol/day) of purine derivatives. The production of rumen microbial N (111 g/day) and the microbial protein yield (699 g/day and 122 g/kg total digestible nutrients) did not differ between the treatments (P > 0.05). Protein-energy supplements (Exp. 2) provided greater ADG (P < 0.001) than MS (Table 5). The ADG increased quadratically (P < 0.001) with an increasing supplementation frequency. The supplementation 5×/week provided higher arroba produced per hectare (9.24) and a higher economic return during the study period (1.64%) compared to the other supplements (Table 6).

Discussion

The increased DM intake without reducing forage intake in animals supplemented with concentrate (0.4% BW) is due the associative or combination effect of supplementation (Oliveira et al. 2015). Supplementation frequency did not impair the intake and digestibility of nutrients. This is due to the ability of ruminants to mitigate the effects of low nutrient supplies

 Table 4
 Nitrogen use pattern of beef cattle supplemented with mineral supplement or concentrate (Exp. 1)

Item	Supplements				SEM	Main effect			Contrasts		
	MS	3×	5×	7×		Treat	Day	Treat × day	$MS \times Freq$	L	Q
Nitrogen intake ^a	132	192	208	204	14	< 0.001	0.999	0.999	< 0.001	0.012	0.015
Excretion of nitrogen in the urine ^a	17	24	28	25	3	0.030	0.002	0.514	0.006	0.772	0.221
Urine urea nitrogen ^a	37	51	60	53	7	0.029	0.002	0.514	0.006	0.771	0.221
Excretion of nitrogen in the faeces ^a	62	78	85	77	4	< 0.001	0.999	0.999	< 0.001	0.590	< 0.001
Serum urea nitrogen ^b	18	27	24	30	3	0.001	0.020	0.349	< 0.001	0.152	0.053
Absorbed nitrogen ^a	70	115	123	128	11	< 0.001	0.999	0.999	< 0.001	0.001	0.435
Nitrogen balance ^a	52	91	95	103	12	< 0.001	0.027	0.817	< 0.001	0.015	0.677

^ag/day

^b mg/dL

 Table 5
 Performance (kg) of

 beef cattle supplemented with

 mineral supplement or

 concentrate (Exp. 2)

	Suppleme	ents		SSEM	Contrasts			
	MS	3×	5×	7×		$MS \times Freq$	L	Q
Initial BW	358	359	359	356	7.21	_	_	_
Final BW	391	417	432	422	3.72	< 0.001	0.364	0.022
Total weight gain	33	59	74	64	3.72	< 0.001	0.364	0.022
Average daily gain	0.396	0.703	0.877	0.762	0.04	< 0.001	0.364	0.022

and to maintain digestibility, even on days without supplementation (De Paula et al. 2011).

The linear reduction in ruminal pH on day 2 by increased supplementation frequency can be explained by the fact that animals $3\times$ and $5\times$ consumed supplement throughout the day due to large quantities supplied, as at the end of day 2, the animals were still consuming supplement. In addition, the lower ruminal pH of receiving the supplement compared to that of MS animals on day 3 was due to higher nutrient concentrations supplied on days 2 and 3, since on day 3, we frequently found remains of supplements $3\times$ and $5\times$.

Beef cattle receiving supplements at low frequencies are effective at maintaining the RAN at levels appropriate

 Table 6
 Economic indicators for

 beef cattle supplemented with
 mineral supplement or

 concentrate (Exp. 2)
 2

for growth and microbial activity (Moraes et al. 2010). This was shown by the higher RAN levels recorded at time 4 of animals $3\times$, $5\times$ and $7\times$ on 3 days of ruminal fluid collection, which was due to the higher supply of nutrients provided by the supplements; for the MS animals, nitrogen intake was lower, resulting in lower nitrogen recycling.

The quadratic behaviour of RAN at time 4 on days 1 and 3 verified that the reduced supplementation frequency indicates that a diet rich in rumen-degradable protein can reduce ruminal nitrogen fluctuations that can occur when large amounts of supplement are provided, as was the case in the treatment with a low-frequency supplementation.

	Supplements ^a							
	MS ^b	3×	5×	7×				
Supplement intake (kg/animal/week)	0.49	10.5	10.5	10.5				
Cost supplementation/animal (US\$/84 days)	2.42	34.69	34.69	34.69				
Cost distribution supplement/animal (US\$) ^c	1.19	3.56	5.93	8.31				
Total cost (US\$) ^d	3.61	38.25	40.62	43.00				
Weight gain (kg/84 days)	32.8	58.2	72.9	66.2				
Gain in equivalent carcass yield (ECY) ^e	1.14	2.02	2.53	2.30				
Total gain in @/ha ^f	4.16	7.38	9.24	8.40				
Cost per arroba produced (US\$) ^g	3.17	18.97	16.08	18.72				
Additional daily BW (ADBW) (kg/day) ^h	_	0.30	0.48	0.40				
Revenue (US\$) per kg of ADBW ⁱ	_	1.42	1.42	1.42				
Return (US\$) of ADBW in 84 days ^j	_	35.97	56.83	47.42				
Economic return in period (US\$) ^k	-	1.04	1.64	1.37				

^a Supplementation (3, 5 and 7×/week)

^b Mineral supplement

^c Time for distribution supplements (0.5 h)/treatments (4)/animals (3) × time man + machine (US\$9.5) × days of supplementation (MS = 1×/week; supplements = 3, 5 and 7 days/week)

^d Cost of supplementation + distribution

^e weight gain × 52% carcass yield/@(15 kg)

^fECY \times number of animals (3)/area (0.82 ha)

^g Total cost/Gain in ECY

^h Additional gain compared to animals MS

ⁱ Price of US\$40.87/arroba × 52% of carcass yield

^jReturn = (CY \times ADBW \times 84 days)

^k Return of ADBW/cost of supplementation; US\$3.16

The quadratic effect on the amount of RAN and no effect on the digestibility of CP indicate that the increased supplementation frequency has led to a greater intake of ruminal nitrogen and may have caused a greater nitrogen leak in the rumen.

To guarantee the fibrolytic ability of the rumen microorganisms is necessary that the supplementation provided of at least 8 mg/dL of RAN (Detmann et al. 2014). Thus, only supplemented animals ($3\times$ —day 2, $5\times$ and $7\times$) had, at time 4, appropriate RAN levels for maximum microbial growth and more efficiently used the energy derived from carbohydrate degradation.

The greater UUN for animals with supplementation frequency was positively correlated to SUN concentrations. The improvement in N use efficiency of ruminants by synchrony between fermentable energy and available N can be verified by the reduction of ENU (Waldrip et al. 2013).

The greater ADG obtained with supplements $5 \times$ enables a more efficient use of the pastures, reduces slaughter age and improves the economic efficiency when compared to traditional systems. A reduction of supplementation can be used as a tool to maximise labour and to reduce production costs (Assad et al. 2015). Furthermore, protein-energy supplementation can accelerate the return on invested capital due to the short time of the growing phase of the animals.

However, lower supplementation frequencies may be a harmful to young animals due to the competition for supplemented food with older and/or heavier animals, causing heterogeneity in the consumption of concentrate and resulting in an unevenness of lots of animals with the same physiological age.

In addition, the supplements $3\times$, $5\times$ and $7\times$ /week resulted in additional weight gain (compared to MS animals) of 307, 481 and 366 g/day, respectively. Thus, supplementation could effectively improve the digestibility of the forage consumed, improving total DM intake and digestible energy (Assad et al. 2015). These additional gains were higher than the daily costs of supplementation. Therefore, although supplementation was associated with considerable costs (US\$39.48 superior to MS animal), all strategies yielded positive economic returns and represent economically viable alternatives to the common supplementation practices during the rainy season in Brazil.

Conclusion

The supplement provided $5 \times$ /week for grazing beef cattle resulted in better nutritional parameters, higher performance and increased financial return, thus demonstrating the potential use of altered supplementation frequencies in intensive beef production systems.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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