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Effects of prepartum supplementation levels on the performance and metabolic responses of Nellore cows in a grazing system

Efeitos dos níveis de suplementação pré-parto sobre o desempenho e respostas metabólicas de vacas Nelore sob sistema de pastejo

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Highlights

Prepartum supplementation increased neutral detergent fiber intake and digestibility.
Our study not evidence differences in average daily gain of beef cows in grazing.
Supplementation increased serum urea nitrogen levels prepartum and postpartum.
Prepartum supplementation increased progesterone concentrations at postpartum.

Abstract

This study aimed to evaluate the effects of prepartum supplementation levels on the productive and nutritional performance and metabolic responses of Nellore cows in a grazing system. Forty-four pregnant Nellore cows multiparous with an initial mean gestation time of 230 days, average initial body weight (BW) of 541 ± 19 kg, and body condition score (BCS) of 5.5 ± 1.7 were used and distributed in a completely randomized design, with four treatments and 11 cows per treatment. The treatments evaluated consisted of four supplementation levels in the prepartum period with a duration of 60 days, according to the following scheme: 0.0 (Control = CON); 0.5 (LOW); 1.0 (Medium = MED) and; 1.5 (High = HIG) kg per animal day^{-1} of supplement. The supplement was composed of wheat meal and urea, and formulated to contain 22% of crude protein (CP). A positive linear effect ($P < 0.05$) was observed on the intake of CP and CP:digested organic matter (DOM) ratio as supplementation levels were increased. In addition, a quadratic effect ($P < 0.05$) was detected on the intake of neutral detergent fiber corrected for

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ash and protein (apNDF), digested neutral detergent fiber, and DOM with increasing supplementation levels in the prepartum period, in which MED cows had higher intakes compared to CON, LOW, and HIG cows. Prepartum supplementation levels did not affect (P > 0.10) the average daily gain in the prepartum or postpartum periods and, BCS of the dams, and BW of calves at birth or 45 days of age. In the prepartum period, supplementation levels did not affect (P > 0.10) the blood concentrations of total proteins, albumin, globulins, glucose, cholesterol, triglycerides, non-esterified fatty acids, and beta-hydroxybutyrate in the experimental cows. In conclusion, the provision of 1.0 kg of supplement per animal per day during the last 60 days of pregnancy improves prepartum nutritional performance and postpartum milk production of grazing beef cows.
Key words: *Bos indicus.* Metabolism. Peripartum period. Ruminant nutrition. Tropical forages.

Resumo

Este estudo objetivou avaliar os efeitos dos níveis de suplementação pré-parto sobre o desempenho produtivo e nutricional e respostas metabólicas de vacas Nelore sob sistema de pastejo. Foram utilizadas 44 vacas Nelore gestantes multíparas, com tempo médio de gestação inicial de 230 dias, peso corporal (PC) médio inicial de 541 ± 19 kg e escore de condição corporal (ECC) de 5,5 ± 1,7, distribuídas em delineamento inteiramente casualizado, com quatro tratamentos e 11 vacas por tratamento. Os tratamentos avaliados consistiram em quatro níveis de suplementação no pré-parto com duração de 60 dias, conforme esquema: 0,0 (Controle=CON); 0,5 (Baixo); 1,0 (Médio=MED) e; 1,5 (Alto=HIG) kg por animal dia^{-1} de suplemento. O suplemento foi composto por farelo de trigo e uréia, e formulado para conter 22% de proteína bruta (PB). Observou-se efeito linear positivo (P < 0,05) no consumo de PB e na relação PB:matéria orgânica digerida (MOD) à medida que os níveis de suplementação foram aumentados. Além disso, foi detectado efeito quadrático (P < 0,05) no consumo de fibra em detergente neutro corrigida para cinzas e proteína (apNDF), fibra em detergente neutro digerida e MOD com o aumento dos níveis de suplementação no período pré-parto, em que vacas MED tiveram uma maior ingestão em comparação com vacas CON, LOW e HIG. Os níveis de suplementação pré-parto não afetaram (P > 0,10) o ganho médio diário nos períodos pré-parto ou pós-parto e ECC das matrizes e, PC dos bezerros ao nascer ou aos 45 dias de idade. No período pré-parto, os níveis de suplementação não afetaram (P > 0,10) as concentrações sanguíneas de proteínas totais, albumina, globulinas, glicose, colesterol, triglicerídeos, ácidos graxos não esterificados e beta-hidroxibutirato nas vacas experimentais. Em conclusão, o fornecimento de 1,0 kg de suplemento por animal por dia durante os últimos 60 dias de gestação melhora o desempenho nutricional pré-parto e a produção de leite pós-parto de vacas de corte em pastejo.
Palavras-chave: *Bos indicus.* Forragem tropical. Metabolismo. Nutrição de ruminante. Período pré-parto.

Introduction

Cattle production in the tropics mainly uses tropical grasses as a basal forage resource. Although such grasses can provide low-cost energy substrates from fibrous carbohydrates (Paulino et al., 2008), they cannot be considered a balanced diet, as they have nutritional limitations that restrict the intake and digestion of forage or the metabolizability of absorbed substrates (Detmann et al., 2014). This is one of the principal reasons for the low reproductive performance of grazing beef cows in tropical conditions (Madureira et al., 2014).

According to Wettemann et al. (2003), several factors affect the reproductive performance of beef cows, but nutrition is perhaps the factor with the highest impact. Nutrition can influence ovarian function as it modulates the secretion of hormones that govern female reproductive processes (Chilliard et al., 2005). In tropical conditions, such as in Brazil, the peripartum of cows may coincide with the season of poor quality and availability of forage, leading to under-nutrition during the period of highest demand (National Academies of Sciences, Engineering, and Medicine [NASEM], 2016) and consequently affecting the growth of the fetus. Thus, supplementation programs are adopted to reduce these nutritional deficiencies, optimize forage intake and nutrient gain, improve metabolic status, maintain body condition scores at the end of pregnancy, and improve reproductive performance (Hess, 2005).

Previous studies during the last trimester of pregnancy have provided evidence that feeding strategies have a positive effect on the productive and metabolic responses of grazing beef cows (Alexander et al., 2002; Silva et al., 2017; Sotelo et al., 2018; Almeida et al., 2020; Moura et al., 2020; Moreno et al., 2023). However, few studies have evaluated the carry-over effects of prepartum supplementation on the performance and metabolism of beef cows.

Therefore, we hypothesized that higher levels of prepartum supplementation would improve the productive and nutritional performance and metabolic status of grazing beef cows. Our objective was to evaluate the effects of prepartum supplementation on Nellore cows within a grazing system.

Material and Methods

Location and weather conditions

The experiment was carried out at the Beef Cattle Farm of the Animal Science Department of Federal University of Viçosa, Viçosa-MG, Brazil (20°45'S, 42°52'W), from August to November, with a duration of 105 days, corresponding to the end of the dry season and the dry-water transition season. The total rainfall during the experiment was 570 mm, and the average temperature was 22.2 °C.

Animals, experimental design, diets, and management

This study was approved by the Brazilian Ethics Committee on Animal Use (CEUAP/UFV - process no. 034/2017), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control (CONCEA).

Forty-four pregnant Nellore cows multiparous with an initial mean gestation time of 230 days, average initial body weight (BW) of 541 ± 19 kg, and body condition score (BCS) of 5.5 ± 1.7 on a scale of 1 to 9 points according to National Research Council [NRC] (1996) were used.

The cows were distributed in a completely randomized design, with four treatments and 11 cows per treatment (an individual cow was considered the experimental unit). The treatments evaluated consisted of four supplementation levels in the prepartum for 60 days, according to the following scheme: 0.0 (Control = CON); 0.5 (LOW); 1.0 (Medium = MED) and; 1.5 (High = HIG) kg per animal day^{-1} of supplement. The supplement was composed of wheat meal and urea, and formulated to contain 22% of crude protein (CP; Table 1), and provided daily at 12:00 am. Levels of 0.5, 1.0, and 1.5 kg per animal day^{-1} of supplement corresponded to approximately 10, 20, and 30% of the CP dietary requirements, respectively, for pregnant Zebu cows with BW of 550 kg and an expected gain of 0.1 kg day^{-1} (Valadares et al., 2016). After calving, the animals of all treatments did not receive any more supplementation, only a mineral mixture.

Table 1
Chemical composition of supplement and forage consumed by the animals during the experimental period

Item[1]	Supplement	Forage[2]	Forage[3]	Forage[4]	Forage[5]	Forage[6]
Chemical composition (g kg DM^{-1})						
Dry matter (g kg^{-1} as-fed)	874.0	590.0 ± 0.02	579.0 ± 0.04	702.0 ± 0.13	323.0 ± 0.03	274.0 ± 0.05
Organic matter	949.0	932.0 ± 0.23	927.0 ± 0.67	984.0 ± 0.40	930.0 ± 0.26	917.0 ± 0.33
Crude protein	239.0	4.1 ± 0.06	39.6 ± 0.94	37.5 ± 0.40	87.7 ± 3.23	91.3 ± 0.35
apNDF	265.0	698.0 ± 1.72	693.0 ± 1.38	702.0 ± 0.44	637.0 ± 4.36	571.0 ± 3.86
Indigestible NDF	86.2	266.0 ± 4.06	261.0 ± 6.04	306.0 ±1.06	297.0 ± 3.52	172.0 ± 1.81

[1]apNDF: neutral detergent fiber corrected for ash and protein residue; NDF: neutral detergent fiber. [2]Forage: Means ± standard error of the mean of sample obtained by hand-plucking in the digestibility trial. [3, 4, 5, 6]Forage: Means ± standard error of the mean samples obtained by hand-plucking during each experimental subperiod.

Animals were submitted to 14 days of adaptation to the diet and experimental conditions. The experiment lasted 105 days, corresponding to 60 days prepartum and 45 days postpartum. The animals were managed on eight 6-ha paddocks of *Urochloa decumbens* Stapf and provided with covered feeders and drinking water. All the animals had unrestricted access to water and a mineral mixture (500 g kg^{-1} $CaHPO_4$; 471.9 g

kg^{-1} NaCl; 15 g kg^{-1} $ZnSO_4$; 7 g kg^{-1} Cu_2SO_4; 500 mg kg^{-1} $CoSO_4$; 500 mg kg^{-1} KIO_3; 100 g kg^{-1} Na_2SeO_3; and 5 g kg^{-1} $MnSO_4$).

Forage samples and nutritional performance

Forage nutritional composition was assessed by hand-plucked samples collected every 30 days. Additionally, a second pasture sample was collected to estimate the total availability of dry matter (DM) and potentially digestible dry matter (pdDM). Subsamples were randomly collected in each paddock by cutting it close to the ground using a metal square (0.5 m × 0.5 m). Samples were oven-dried at 55 °C for 72 h. After drying, the samples were ground in a knife Willey mill (TE-680, SOLAB®) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen and stored for later analysis.

On 45 days before the expected date of calving, a 9-day digestibility trial was carried. From the first to the eight day of the trial chromium oxide (Cr_2O_3) was used as an external marker to estimate fecal excretion (20 g per animal day^{-1}). The first six days were used for the animal's adaptation to the Cr_2O_3 (stabilization of marker excretion). The indigestible neutral detergent fiber (iNDF) was used as an internal marker to estimate forage DM intake. Fecal samples were collected immediately after defecation or taken directly from the rectum of animals (at amounts of approximately 200 g) on the last four days of the trial at different times according to the following schedule: day 6 - 18h00, day 7 - 14h00, day 8 - 10h00, and day 9 - 06h00. Fecal samples were identified, oven-dried at 55 °C for 72 h, and ground as previously described.

Productive performance

At the beginning (60 d before calving), both at calving, and end of experiment (45 d after calving), the animals were weighed to determine the average daily gain (ADG) and final BW (FBW). Calf BW was recorded at birth and at 45 days after calving. The BWs were obtained at 06h00, except on the day of calving. The cows BW was corrected to shrunk BW, according to Gionbelli et al. (2015), to avoid the possible confounding effect of the last meal filling the digestive tract:

$$SBW = 0.8084 \times BW^{1.0303}$$

Where, SBW was the shrunk body weight (kg) and BW was the body weight (kg).

In parallel with weighing, the BCS was assessed by three evaluators on a scale of 1 to 9 (NRC, 1996).

The cows were milked on 30 d and 45 d postpartum to estimate the production and composition of milk according to procedures described by Moreno et al. (2023).

Blood samples and measurements

45 days before the expected date of calving, at calving, 15 d, 30 d, and 45 d after calving, blood samples were collected to quantify the concentrations of urea, total proteins, albumin, glucose, triglycerides, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (βHB), and progesterone. Samples were collected at 07h00 via jugular venipuncture before the supplement offer using vacuum tubes with sodium fluoride and EDTA as a glycolytic inhibitor and anticoagulant (BD Vacutainer® Fluoride/EDTA, São Paulo, Brazil) for glucose

analysis and vacuum tubes with clot and separator gel (BD Vacuntainer®, SST II Advance, São Paulo, Brazil) for the other analyzes. Blood samples were centrifuged at 3600 × g for 15 minutes, and the serum and plasma were subsequently frozen at -20 ºC for further analysis.

Analytical procedures

Samples of forage, feces, and supplements ground to 1 mm were analyzed for DM (dried overnight at 105 °C; method INCT-CA G-03/1), ash (complete combustion in a muffle furnace at 600 °C for 4 h; method INCT-CA M-001/1), CP (Kjeldahl procedure; method INCT-CA N-001/1), ether extract (EE - Randall procedure; method INCT-CA G-005/1), NDF (method INCT-CA F-002/1) corrected for ash (NDIA - neutral detergent insoluble ash; method INCT-CA M-002/1) and protein (NDIP - neutral detergent insoluble protein; method INCT-CA N-004/1), residue (apNDF; using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein), according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA) (Detmann et al., 2012). The content of iNDF in samples of feces, forage, and supplement (ground through 2-mm sieves) was estimated as the residual NDF remaining after 288 h of ruminal *in situ* incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY) according to Detmann et al. (2012).

Feces samples were also analyzed for chromium concentration by nitroperchloric digestion and atomic absorption spectrophotometry (method INCT-CA M-005/1 according to Detmann et al. (2012).

The fecal DM excretion was estimated using the chromic oxide marker, based on the ratio between the amount of chromium supplied and its concentration in the feces (Detmann et al., 2016).

Individual supplement intake (ISI) was estimated by the ratio between the amount of supplement provided and the number of animals in the group. Individual DM intake (DMI) was estimated by using iNDF as an internal marker (Detmann et al., 2016).

The pdDM in forage available on pasture was estimated using the following equation described by Paulino et al. (2008):

$$pdDM = 0.98 \times (100 - NDF) + (NDF - iNDF)$$

Milk fat, protein, lactose, and total solids contents were analyzed using infrared spectrophotometry (Foss MilkoScan FT120, Hillerød, Denmark). Milk production was corrected to 4% of fat ($Milk_{4\%}$) according to NRC (2001):

$$Milk_{4\%}\ (kg) = 0.4 \times (milk\ production) + [15 \times (fat\ production \times milk\ production/100)]$$

Blood urea (K056), glucose (K082) and triglycerides (K117, Bioclin® Quibasa, Belo Horizonte, Brazil) concentrations were quantified by enzymatic-colorimetric methods. Total proteins (K031) and albumin (K040, Bioclin® Quibasa, Belo Horizonte, Brazil) by colorimetric methods in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). Blood NEFA (FA115) by colorimetric method and βHB (RB1007, Randox® Laboratories Ltd., Antrim, UK) by enzymatic method. Globulins concentrations were calculated as the difference between the analyzed total proteins and albumin, and serum urea nitrogen (SUN) was estimated as 46.67% of the total serum urea. All metabolites were analyzed using an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). The progesterone concentrations (33550, Beckman Coulter®, Brea, USA) were analyzed by indirect chemiluminescence method using the Access® 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical analyses

The experiment was conducted and analyzed in a completely randomized design with a duple error structure. The MIXED procedure of the SAS 9.4 (Statistical Analysis System, Inc., Cary, NC, USA) was used for all statistical analyses. The comparisons between the means of the treatments were carried out by means of orthogonal contrasts, relative to the effects of linear, quadratic, and cubic order in function the level of supplement offered in prepartum, according to the following mathematical model:

$$Y_{ijk} = \mu + T_i\,(0,1,2,3) + e_{(i)j} + \varepsilon_{(ij)k}$$

where, Y_{ijk} = observations of individual k on paddock j under treatment i; μ = overall mean; T_i = fixed effect of treatment, being 0, 1, 2, 3 = 0.0 (Control = CON); 0.5 (LOW); 1.0 (Medium = MED) and; 1.5 (High = HIG) kg per animal day^{-1} of supplement in the prepartum; $e_{(i)j}$ = random error, unobservable, associate to each j paddock under treatment i, assumed to be normally and independently distributed (NID; 0, σe^2); and $\varepsilon_{(ij)k}$: random error, unobservable, associate to each k observation on j paddock under treatment i, assumed to be NID (0, σe^2). The choice of the best (co) variance matrix was performed following the Akaike information criteria with correction. The degrees of freedom were estimated according to the Kenward-Roger method. The normality of the residuals was verified by the Shapiro-Wilk test and the homogeneity of the variances by the Bartlett test. Initial BW and BCS were used as covariates for analyzes related to ADG, BW at calving, FBW and BCS measurements. The blood metabolites and progesterone, and milk yield were evaluated as repeated measures over time. Statistical significance was considered when $P \leq 0.05$, and tendences were considered at $0.05 < P \leq 0.10$.

Results and Discussion

The average availability of DM and pdDM was 3856 and 2535 kg ha^{-1}, respectively. The forage consumed by the animals presented an average of 38.6 and 89.5 g of CP kg^{-1} DM in the prepartum and postpartum periods, respectively (Table 1). The forage consumed by cows during the prepartum period (Table 1), presented a much lower value of CP than the 70-80 g of CP kg^{-1} DM suggested by Lazzarini et al. (2009) as the minimum for using fibrous carbohydrates and optimizing forage use. However, at postpartum period, the forage presented a value of CP that was very close to that suggested by Lazzarini et al. (2009).

Regarding voluntary intake (kg day^{-1}), a positive linear effect ($P < 0.05$) was observed on CP and CP:DOM ratio as supplementation levels increased. In addition, a quadratic effect ($P < 0.05$) was detected on the intake of apNDF, digested NDF (DNDF), and DOM with increasing supplementation levels in the prepartum period, in which MED cows had higher intakes compared to CON, LOW, and HIG cows (Table 2). Likewise, a trend towards quadratic effect ($P = 0.053$) was observed, in which MED cows had a higher intake of total DM and organic matter (OM), whereas LOW cows showed a higher intake of forage DM. When evaluating intake as g kg^{-1} BW, a quadratic effect ($P < 0.05$) was verified on total DM, forage DM, OM, and apNDF, where LOW cows had higher intakes than CON, MED, and HIG cows.

Table 2
Voluntary intake and total digestibility of Nellore cows in grazing system receiving prepartum supplementation levels

Item[1]	Treatments[2]				SEM[3]	P-value[4]		
	CON	LOW	MED	HIG		L	Q	C
Intake, kg day^{-1}								
Total DM	6.43	9.68	9.73	8.97	0.720	0.079	0.051	0.501
Forage DM	6.43	9.24	8.85	7.66	0.721	0.371	0.051	0.501
Organic matter	6.00	8.99	9.08	8.41	0.666	0.074	0.053	0.514
Crude protein	0.23	0.50	0.59	0.64	0.041	0.002	0.064	0.480
apNDF	4.60	7.33	7.47	7.02	0.524	0.036	0.040	0.438
Indigestible NDF	1.82	2.59	2.69	3.05	0.399	0.102	0.646	0.632
DNDF	2.49	4.48	4.75	4.08	0.358	0.036	0.021	0.652
DOM	2.59	4.50	4.95	4.00	0.357	0.043	0.016	0.968
CP:DOM	87.6	111.5	119.1	155.2	6.330	0.002	0.392	0.188
Intake, g kg^{-1} BW								
Total DM	12.36	17.74	17.30	16.73	0.764	0.025	0.020	0.177
Forage DM	12.36	16.94	15.65	14.26	0.777	0.299	0.021	0.179
Organic matter	11.52	16.49	16.15	15.63	0.648	0.018	0.016	0.159
apNDF	8.76	13.48	13.30	13.14	0.530	0.007	0.012	0.114
Indigestible NDF	3.48	4.70	4.81	5.75	0.801	0.128	0.870	0.616
Digestibility, g g^{-1}								
Organic matter	0.440	0.500	0.545	0.488	0.890	0.011	0.003	0.159
Crude protein	0.157	0.275	0.369	0.417	5.490	0.002	0.026	0.297
apNDF	0.540	0.611	0.631	0.576	1.570	0.179	0.016	0.733
DOM, g kg^{-1} DM	411	465	508	460	7.200	0.005	0.002	0.917

[1]DM: dry matter; apNDF: neutral detergent fiber corrected for ash and protein residue; DNDF: digested neutral detergent insoluble fiber; DOM: digested organic matter; CP:DOM: crude protein and DOM ratio. [2]CON: unsupplemented beef cows; LOW: supplemented beef cows with 0.5 kg per animal day^{-1}; MED: supplemented beef cows with 1.0 kg per animal day^{-1}; HIG: supplemented beef cows with 1.5 kg per animal day^{-1}. [3]SEM: standard error of means. [4]L, Q and C: effects of linear, quadratic and cubic order according to supplementation levels.

Some studies on cattle fed low-quality tropical forage have suggested that protein supplementation can increase forage intake (Lazzarini et al., 2009; Sampaio et al., 2010; Detmann et al., 2014) because protein supplementation stimulates the growth of fibrolytic bacteria (Russel, 2002) and increases the digestibility of fibrous carbohydrates. Sampaio et al. (2010) suggested that the dietary CP content must be near 100 g CP kg^{-1} DM, optimizing forage intake. In this sense, the higher intake of apNDF and DNDF from pasture observed in MED cows demonstrates that, up to this level, supplementation with nitrogenous compounds optimized the ruminal environment and the action of fibrolytic bacteria on the fiber of forage, thus producing a larger, effectively degraded, NDF fraction.

The dietary protein-energy ratio is one of the leading indicators for understanding the metabolic effects of protein on the voluntary forage intake of cattle in tropical pastures. The maximum forage intake is observed with a dietary CP:DOM ratio of 210 g kg^{-1} (Poppi & McLennan, 1995). However, in this study, the dietary CP:DOM ratios for unsupplemented and supplemented cows were, on average, 87.6 and 128.6 g kg^{-1}, respectively. Thus, both groups had an unbalanced dietary protein-to-energy ratio considering intake adequacy. Nonetheless, positive linear effect on the CP:DOM ratio was observed with increasing supplementation levels, which appears to support at trend of higher forage intake in supplemented animals.

The OM and DOM showed maximum digestibility at 1.0 kg per animal per day in MED cows, along with a higher total DM intake and fiber digestibility. The increase in supplementation to 1.5 kg per animal per day in HIG cows seems to have impaired fiber digestibility, and perhaps this explains the drop in intake observed at this level. The CP digestibility pattern seems to directly reflect the increase in highly degradable protein available in the diet. According to Van Soest (1994), the increase in CP intake promotes a lower participation of the endogenous protein and reduces the representativeness of the fecal metabolic fraction of nitrogenous compounds.

Prepartum supplementation levels did not affect ($P > 0.10$) the ADG in the prepartum or postpartum periods, BW at calving, or final BW (45 days postpartum; Table 3). Likewise, there was no effect ($P > 0.10$) of supplementation levels on the BCS of cows at calving and the end of the experiment. Supplementation levels in the prepartum period did not influence ($P > 0.10$) the BW of calves at birth or 45 days of age.

Table 3
Productive performance in prepartum and postpartum of Nellore cows in grazing system receiving prepartum supplementation levels

Item[1]	Treatments[2]				SEM[3]	P-value[4]				
	CON	LOW	MED	HIG		L	Q	C	COL	TREAT × COL
Prepartum										
Average daily gain, kg day^{-1}	0.286	0.432	0.560	0.444	0.208	0.560	0.565	0.820	-	-
Calving										
BW, kg	557.0	563.0	574.0	572.0	8.10	0.171	0.646	0.642	-	-
Body condition score	5.5	5.6	5.3	5.5	0.14	0.905	0.944	0.220	-	-
Calf BW, kg	34.1	34.3	37.3	36.6	1.580	0.196	0.756	0.371	-	-
45 d postpartum										
Average daily gain, kg day^{-1}	-0.099	0.073	-0.005	-0.027	0.106	0.785	0.404	0.540	-	-
Final BW, kg	513.0	508.0	525.0	509.0	9.70	0.912	0.606	0.233	-	-
Body condition score	5.4	5.0	5.2	5.3	0.11	0.884	0.116	0.198	-	-
Calf BW, kg	71.8	80.8	85.5	80.7	3.09	0.104	0.101	0.742	-	-
$Milk_{4\%}$, kg day^{-1}	8.04	9.53	10.28	8.76	0.465	0.185	0.003	0.474	0.900	0.033
Fat, %	4.79	4.87	4.89	4.35	0.319	0.457	0.405	0.755	0.612	0.227
Protein, %	3.14	3.02	3.03	2.93	0.073	0.174	0.873	0.502	0.015	0.103
Lactose, %	4.72	4.66	4.68	4.77	0.053	0.546	0.209	0.970	0.101	0.346
Total solids, %	13.80	13.50	13.60	13.40	0.360	0.591	0.938	0.701	0.227	0.352

[1]BW: body weight; $Milk_{4\%}$: milk production corrected to 4% of fat. [2]CON: unsupplemented beef cows; LOW: supplemented beef cows with 0.5 kg per animal day^{-1}; MED: supplemented beef cows with 1.0 kg per animal day^{-1}; HIG: supplemented beef cows with 1.5 kg per animal day^{-1}. [3]SEM: standard error of means. [4]L, Q and C: effects of linear, quadratic and cubic order according to supplementation levels; COL: collection day effect; TREAT × COL: treatment collection day interaction effect.

An interaction effect ($P < 0.05$) was observed between treatment and collection days on milk production corrected to 4% of fat content (Milk4%) (Table 3). Examination of this interaction indicates that, in the CON cows, milk production varied over collection days, being higher in the first collection ($P < 0.05$; Figure 1). In addition, in the second collection (45 d), the LOW, MED, and HIG cows showed higher milk4% production than CON cows ($P < 0.05$). On the other hand, a quadratic effect ($P < 0.05$) was observed between the treatments groups for milk4% production, where MED cows showed higher values compared to the CON, LOW, and HIG cows. Prepartum supplementation levels did not affect ($P > 0.10$) the concentration of fat (average 4.73%), protein (average 3.03%), lactose (average 4.71%), or total solids (average 13.58%) in the milk.

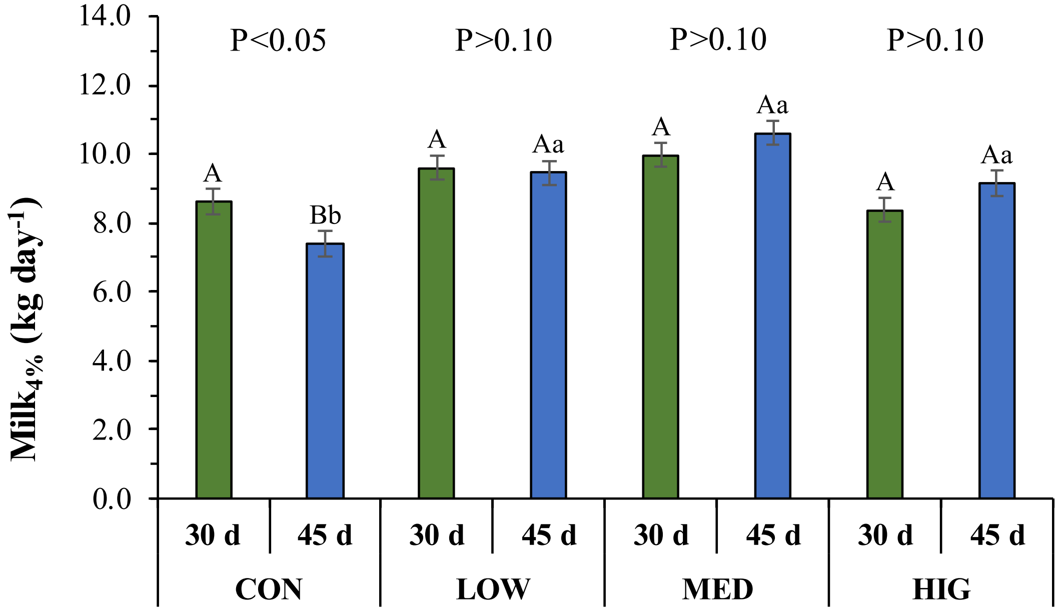

Figure 1. Milk$_{4\%}$ production of different treatments according to collection days. CON: unsupplemented beef cows; LOW: supplemented beef cows with 0.5 kg per animal day^{-1}; MED: supplemented beef cows with 1.0 kg per animal day^{-1}; HIG: supplemented beef cows with 1.5 kg per animal day^{-1} of supplement in the prepartum. Mean of treatments × collection day without a common capital letter or days within each treatment without a common lowercase letter differ significantly at P < 0.05. Error bars represent SEM.

The ADG of cows in the prepartum period and calf BW at 45 d showed an average differential gain in supplemented animals compared to the unsupplemented ones, by an average of 0.192 kg day^{-1} and 10.5 kg, respectively. The highest response was observed in MED cows (0.274 kg day^{-1} and 13.7 kg), compared to LOW (0.146 kg day^{-1} and 9 kg) and HIG (0.158 kg day^{-1} and 8.9 kg) cows. These results show the positive effects of prepartum supplementation in grazing cows.

However, supplementation did not promote sufficient changes to favor fat deposition in the prepartum and postpartum periods, which would explain the similar BCS evidenced in the cows (Table 3). This finding can be attributed to the animals' BCS being adequate (>5.0) throughout the experimental period. Silva et al. (2017) and Moura et al. (2020) did not observe an increase in BCS in pregnant cows supplemented in grazing pastures.

Previous studies in cattle have provided evidence that feeding strategy during the last trimester of gestation can alter offspring birth weight, suggesting that the maternal dietary energy source affects fetal growth (Radunz et al., 2010, 2011). Marques et al. (2016) suggested that females who maintain an adequate BCS during pregnancy do not have impaired offspring birthweight, which might explain the similar calf BW at birth in the present study. Several studies found no difference in calf BW following prepartum supplementation (Summers et al., 2015; Silva et al., 2017; Moreno et al., 2023).

The higher $milk_{4\%}$ yield at 45 d for the cows receiving prepartum supplementation can be explained by the more significant contribution of nutrients via supplement, which promoted an average differential gain of 0.192 kg day^{-1} compared to unsupplemented cows, with a maximal response of 0.274 kg day^{-1} for MED cows, which had the highest milk4% production. Thus, this rich supply of nutrients may have promoted a longer persistence of lactation in supplemented than unsupplemented cows (Table 3). According to Ferreira et al. (2021), greater milk production of cows may be due to larger net energy balance for milk production and mammary glands with higher metabolic activity, although these underlying mechanisms are poorly understood and were not evaluated in the present study. The greater milk4% yield for LOW, MED, and HIG cows compared to CON cows was insufficient to promote greater calf BW at 45 d, even with a differential increase in final BW of 13.7 kg in calves from MED cows.

In the prepartum period, supplementation levels did not affect (P > 0.10) the blood concentrations of total proteins, albumin, globulins, glucose, cholesterol, triglycerides, NEFA, and βHB in the experimental cows (Table 4). However, the SUN showed a quadratic effect (P < 0.05), in which HIG cows had higher levels compared to CON, LOW, and MED cows. On the other hand, in the postpartum period, there was a significant interaction (P < 0.05) between treatment and collection day for SUN, albumin, and cholesterol (Table 4). A closer examination of this interaction indicated that SUN concentration was higher on the first collection day (0 d, at calving) in all treatments compared with the collection at 15, 30, and 45 d postpartum (P< 0.01; Figure 2a). Examination of the interaction for albumin concentration indicated that all treatments showed higher concentrations on the first collection day (0 d, at calving) compared to other collection days (P < 0.05; Figure 2b). Finally, evaluation of the interaction effect for cholesterol concentration indicated that all treatments showed their lowest values in the first collection (P < 0.01; Figure 2c).

The postpartum blood glucose and progesterone concentrations had (respectively) negative and positive linear trend effects (P = 0.086) with increasing supplementation levels. However, there was no effect (P > 0.10) of prepartum supplementation levels on blood concentrations of total proteins, globulins, triglycerides, NEFA, and βHB in the experimental cows (Table 4). Finally, a main effect of collection days (P < 0.05) was observed on blood concentrations of total proteins (Figure 3a), globulins (Figure 3b), glucose (Figure 3c), triglycerides (Figure 3d), NEFA (Figure 3e), βHB (Figure 3f), and progesterone (Figure 3g).

In the prepartum period, the average SUN concentrations for CON and supplemented cows were 8.39 and 13.42 mg dL^{-1}, respectively. This pattern is due to higher CP intake among supplemented animals. The absence of between-treatment differences in glucose, cholesterol, triglycerides, albumin, total proteins, NEFA, and βHB blood concentrations (Table 4) suggests that diets resulted in similar energetic and protein statuses.

Table 4
Metabolic responses in prepartum and postpartum of Nellore cows in grazing system receiving different prepartum supplementation levels

Item[1]	Treatments[2]				SEM[3]	P-value[4]							
						Prepartum			Postpartum				
	CON	LOW	MED	HIG		L	Q	C	L	Q	C	COL	TREAT × COL
Prepartum													
SUN (mg dL^{-1})	8.38	12.31	13.93	14.01	1.123	0.002	0.030	0.768	-	-	-	-	-
Total proteins (g dL^{-1})	6.91	7.39	7.40	7.17	0.171	0.411	0.127	0.801	-	-	-	-	-
Albumin (g dL^{-1})	3.54	3.52	3.44	3.40	0.060	0.130	0.892	0.697	-	-	-	-	-
Globulins (g dL^{-1})	3.31	3.87	3.97	3.71	0.183	0.228	0.103	0.908	-	-	-	-	-
Glucose (mg dL^{-1})	53.63	53.50	54.57	55.38	1.100	0.291	0.703	0.780	-	-	-	-	-
Cholesterol (mg dL^{-1})	140.10	140.30	147.30	127.60	13.87	0.661	0.522	0.619	-	-	-	-	-
Triglycerides (mg dL^{-1})	43.66	47.13	43.75	40.21	4.778	0.564	0.509	0.769	-	-	-	-	-
NEFA (mmol L^{-1})	0.148	0.293	0.145	0.166	0.055	0.737	0.343	0.140	-	-	-	-	-
βHB (mmol L^{-1})	0.403	0.422	0.444	0.388	0.076	0.950	0.656	0.824	-	-	-	-	-
Postpartum													
SUN (mg dL^{-1})	13.00	12.70	16.00	13.20	3.750	-	-	-	0.649	0.520	0.279	0.001	0.004
Total proteins (g dL^{-1})	6.96	7.19	7.30	7.18	0.178	-	-	-	0.433	0.411	0.925	0.001	0.682
Albumin (g dL^{-1})	3.46	3.35	3.42	3.31	0.067	-	-	-	0.324	0.994	0.336	0.001	0.039
Globulins (g dL^{-1})	3.50	3.82	3.91	3.85	0.198	-	-	-	0.318	0.429	0.944	0.001	0.691
Glucose (mg dL^{-1})	76.20	73.60	69.00	68.90	3.080	-	-	-	0.085	0.711	0.656	0.001	0.914
Cholesterol (mg dL^{-1})	134.00	130.00	142.00	118.00	9.900	-	-	-	0.506	0.390	0.335	0.001	0.014
Triglycerides (mg dL^{-1})	22.00	23.20	20.30	20.80	0.970	-	-	-	0.176	0.714	0.111	0.028	0.404
NEFA (mmol L^{-1})	0.239	0.364	0.293	0.304	0.037	-	-	-	0.502	0.161	0.121	0.001	0.674
βHB (mmol L^{-1})	0.430	0.500	0.500	0.471	0.037	-	-	-	0.572	0.336	0.835	0.011	0.231
Progesterone (ng dL^{-1})	1.42	1.92	1.72	3.52	0.441	-	-	-	0.086	0.385	0.272	0.044	0.123

[1]SUN: serum urea nitrogen; NEFA: non-esterified fatty acids; βHB: beta-hydroxybutyrate. [2]CON: unsupplemented beef cows; LOW: supplemented beef cows with 0.5 kg per animal day^{-1}; MED: supplemented beef cows with 1.0 kg per animal day^{-1}; HIG: supplemented beef cows with 1.5 kg per animal day^{-1}. [3]SEM = standard error of means. [4]L, Q and C = effects of linear, quadratic and cubic order according to supplementation levels; COL: collection day effect; TREAT × COL: treatment × collection day interaction effect.

According to González (1997), there is a physiological increase in the concentration of blood SUN toward the end of pregnancy that decreases shortly before and shortly after calving, even in cows with adequate CP dietary content. This increase may explain the higher concentration of SUN at calving (0 d) observed in animals of all treatments groups from collections made at 15, 30, and 45 d after calving (Figure 2a).

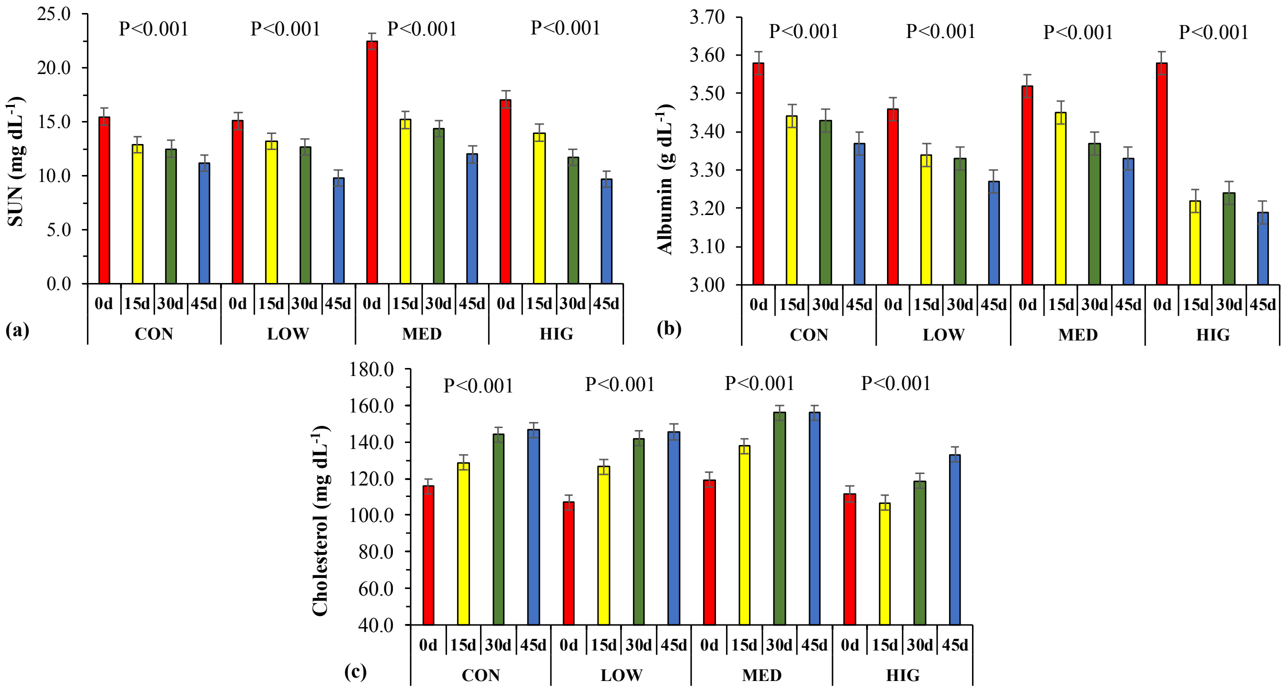

Figure 2. Serum urea nitrogen (SUN) (a), blood albumin (b), and cholesterol (c) concentration of different treatments according to collection days in postpartum. CON: unsupplemented beef cows; LOW: supplemented beef cows with 0.5 kg per animal day^{-1}; MED: supplemented beef cows with 1.0 kg per animal day^{-1}; HIG: supplemented beef cows with 1.5 kg per animal day^{-1} of supplement in the prepartum. Mean of treatments × collection day differ significantly at $P < 0.05$. Error bars represent SEM.

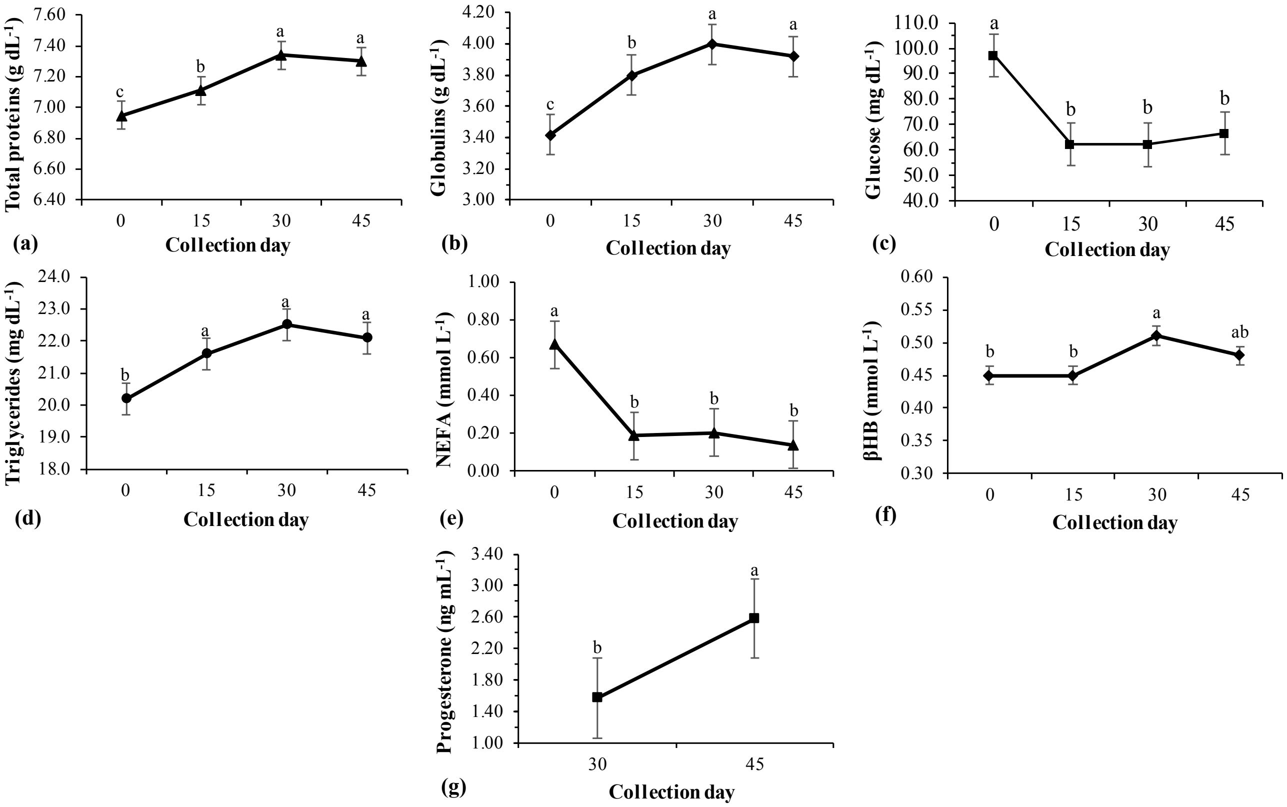

Figure 3. Blood total proteins (a), globulins (b), glucose (c), triglycerides (d), NEFA (e), βHB (f), and progesterone (f) concentrations during the postpartum. [1]Means over the line followed by different letters differ (P < 0.05)

Cows in the postpartum period tend to experience decreases in blood albumin due to the demand for amino acids for protein synthesis in milk, which reduces the synthesis of other proteins in the liver (P. A. Contreras, 2000). This observation may explain the pattern of albumin concentration in the postpartum period in this study (Figure 2b). Across the study, the average albumin concentration was 3.42 g dL^{-1}, within the range of normal levels (2.5 - 3, 6 g dL^{-1}) in cows (Smith, 2006).

Cows in all the treatments groups increased blood cholesterol concentration in the postpartum period (Figure 2c), possibly due to a gradual increase in dry matter intake and homeorhetic changes during lactation (Bertoni & Trevisi, 2013; Ferreira et al., 2021). Cholesterol levels also follow this pattern in beef cows, regardless of the nutritional strategy applied, due to the higher need for lipoproteins to carry triglycerides to the mammary gland. The increase in this study is related to lower levels of triglycerides in early lactation (day 0 of this study; Figure 3d) since they are used to provide energy for lactation and as sources of fatty acids for milk fat synthesis, due to depressed dry matter intake (Aeberhard et al., 2001; Ferreira et al., 2021). According to Puppione (1978), HDL concentrations are higher than the other lipoproteins during this period, possibly due to increased synthesis or VLDL catabolism by mammary tissue.

The decrease in blood concentration of total proteins (Figure 3a) and globulins (Figure 3b) at calving (0 d) and in the following days is due to the transfer of immunoglobulins to the mammary gland for the synthesis of colostrum (Saut, 2008). Saut (2008) found a gradual postpartum increase to normal levels, as observed in the present study (Figures 3a and 3b). Despite daily variations, the total proteins values found in the present study remained within reference levels for cattle throughout the study, between 6.8 and 8.6 g dL^{-1} (Smith, 2006).

The negative linear effect observed in the blood glucose concentration with the increase of prepartum supplementation levels (Table 4) may be related to the higher milk production promoted by prepartum supplementation and the absence of postpartum supplementation. On the other hand, the higher blood glucose concentration on the calving day (0 d) might can be caused by the stress of calving; glycogen catabolism is stimulated in order to minimize stress, while glucocorticoids promote hepatic and muscle gluconeogenesis and decrease glucose uptake and utilization in adipose tissue. Likewise, this physiological response may explain the higher glucose concentrations at calving (0 d), and the decrease in its concentration after calving (Figure 3c) can be explained by the increased use of glucose by the mammary gland for milk production.

The NEFA and βHB are indicators associated with the rate of mobilization of lipid reserves under conditions of negative energy balance. Additionally, the observed NEFA and βHB concentrations suggest that the intensity of mobilization of body reserves was similar between the treatments (Mulliniks et al., 2013; Astessiano et al., 2014). According to G. A. Contreras and Sordillo (2011), the onset of lactation is considered a critical period due to negative energy balance (NEB); however, NEFA concentration is not high in this period. Plasma levels of NEFA are low at the end of lactation and in the dry period but rise two weeks before calving. A peak between calving and ten days postpartum may explain the higher concentration of NEFA at calving (0 d) in relation to the other collection days (15, 30, and 45 d after calving; Figure 3d). Factors present during calving, such as the costly energy demands of parturition and elevated cortisol levels, may be directly associated with higher mobilization in this period. On the other hand, the increase in the blood concentration of βHB up to 30 days after calving (Figure 3f) coincides with blood glucose levels, which are probably due to the reduction in food intake and higher energy demands of milk production, which uses glucose for lactose production.

The positive linear increase of blood progesterone concentration (Table 4) may be due to a carry-over effect of greater intake and digestibility of FDM, CP, and DOM (energy-rich compounds) in LOW, MED, and HIG cows, promoting an increase in progesterone synthesis and suggesting higher luteal activity (Martin et al., 2010). Corroborating this fact, in this study, progesterone values of 1.57 and 2.58 ng mL^{-1} were obtained for 30 and 45 d postpartum, respectively (Figure 3g).

Conclusion

The provision of 1.0 kg of supplement per animal per day during the last 60 days of pregnancy improves prepartum nutritional performance and postpartum milk production of grazing beef cows.

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Conflicts of interest

The authors declare no conflicts of interest.

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