

Effects of protein supplementation on metabolism and performance of grazing Nellore cows under different parity orders

Efeitos da suplementação proteica no metabolismo e desempenho de vacas Nelore em pastejo sob diferentes ordens de parto

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Highlights

Multiparous Nellore cows have greater body mobilization at calving.

Non-supplemented primiparous cows have the worst blood protein status.

Postpartum supplementation improves the performance of grazing Nellore cows.

Abstract

The objective was to evaluate the interaction between the response to supplementation of multiparous and primiparous beef cows on pasture on performance and blood metabolism. Forty Nellore cows were used. The experimental design was completely randomized, in a 2 × 2 factorial arrangement, where two parity orders were evaluated: primiparous (P1) and multiparous (P2), and two postpartum supplementation strategies, as follows: unsupplemented (N), and supplemented (S). Overall, supplementation trend increased weight gain of cows ($P < 0.09$), regardless of parity order, but did

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not alter body condition score (BCS) ($P > 0.20$). In addition, supplementation increased calf weight at 42 days of age ($P < 0.01$), and milk protein and lactose concentrations ($P < 0.03$). On the other hand, multiparous cows had higher milk production ($P < 0.01$), and their calves had higher average dairy gain (ADG) ($P < 0.04$). There was interaction between postpartum day and parity order ($P < 0.03$), in which multiparous cows had higher non-sterified fatty acid (NEFA) in blood at calving. There was an interaction between calving order and supplementation on blood albumin ($P < 0.05$), with lower values for non-supplemented primiparous cows. In addition, supplementation increased insulin like growth factor (IGF-1) in the animal's blood ($P < 0.01$). Postpartum supplementation improves the productive performance of cows. In addition, multiparous cows have greater body mobilization at calving and non-supplemented primiparous cows have the worst blood protein status.

Key words: Calves. IGF-1. NEFA. Tropical pastures. Zebu.

Resumo

O objetivo foi avaliar a interação entre a resposta à suplementação de vacas de corte multíparas e primíparas mantidas em pasto sobre o desempenho e o metabolismo sanguíneo. Foram utilizadas 40 vacas Nelore. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2×2 , no qual foram avaliadas duas ordens de paridade: primíparas (P1) e multíparas (P2), e duas estratégias de suplementação pós-parto, a saber: não suplementadas (N) e suplementadas (S). De modo geral, a suplementação tendeu a aumentar o ganho de peso das vacas ($P < 0,09$), independentemente da ordem de paridade, mas não alterou o escore de condição corporal (ECC) ($P > 0,20$). Além disso, a suplementação aumentou o peso dos bezerros aos 42 dias de idade ($P < 0,01$), bem como as concentrações de proteína e lactose no leite ($P < 0,03$). Por outro lado, as vacas multíparas apresentaram maior produção de leite ($P < 0,01$), e seus bezerros obtiveram maior ganho médio diário (GMD) ($P < 0,04$). Houve interação entre o dia pós-parto e a ordem de paridade ($P < 0,03$), na qual as vacas multíparas apresentaram maiores níveis sanguíneos de ácidos graxos não esterificados (AGNE) no momento do parto. Observou-se interação entre a ordem de paridade e a suplementação em relação à albumina sanguínea ($P < 0,05$), com valores inferiores para as vacas primíparas não suplementadas. Adicionalmente, a suplementação aumentou os níveis sanguíneos do fator de crescimento semelhante à insulina (IGF-1) nos animais ($P < 0,01$). A suplementação pós-parto melhora o desempenho produtivo das vacas. Além disso, as vacas multíparas apresentam maior mobilização corporal no momento do parto, enquanto as vacas primíparas não suplementadas demonstram o pior estado proteico sanguíneo.

Palavras-chave: Bezerros. IGF-1. NEFA. Pastos tropicais. Zebu.

Introduction

The postpartum period of primiparous beef females is challenging due to high nutritional requirements, compromising performance during the breeding season and the weaning weight of their calves. Cows

experiencing their first calving are therefore in a different metabolic state than multiparous cows (Coffey et al., 2006), as they require nutrients for their continued growth and the development of their calf. This situation is further increased in beef cows raised on pasture, as grass rarely offers a balanced diet

for these animals (Reis et al., 2020). Adequate nutrition is crucial for primiparous females to avoid the premature culling of cows with high genetic potential due solely to inadequate nutritional management

Indeed, Ferreira et al. (2021) demonstrated that blood indicators of protein status during lactation are directly influenced by parity order. Primiparous females exhibit more unbalanced metabolic and hormonal profiles, accompanied by a greater body condition loss. Similarly, Cattaneo et al. (2023) reported that primiparous dairy cows display a distinct metabolic and endocrine status compared with multiparous cows. Several studies have evaluated the effects of postpartum nutrition on the metabolism of dairy cows with different parity orders (Nasrollahi et al., 2017; Neave et al., 2018). However, regarding Zebu cows under grazing, to the best of our knowledge, no studies have evaluated the productive and metabolic responses of cows with different parity orders to supplementation. Although some research has compared the performance and metabolism of Nellore cows of different parity orders in the postpartum, all animals received protein supplementation. Therefore, it remains unclear whether cows of different parity orders respond differently to supplementation in terms of productive performance and metabolic adaptations.

Understanding the metabolic patterns of multiparous and primiparous cows under different nutritional plans is essential to avoid the negative effects of homeorhetic processes (e.g., lactation) on animal performance. In addition, adequate nutritional management is particularly important for primiparous cows to avoid the premature culling of

females with high genetic potential due to nutritional limitations. We hypothesized that postpartum supplementation would result in greater productive and metabolic benefits in primiparous cows than in multiparous cows. Therefore, this study aimed to evaluate the effects of postpartum supplementation on productive performance and metabolic status of Nellore cows of different parity orders managed under grazing conditions.

Material and Methods

The experiment was conducted at the Universidade Federal de Vicosa, Vicosa, MG, Brazil (20° 45' S, 42° 52' W). During this period, the average temperature and rainfall were 21.5° C and 557.6 mm (INMET- Viçosa Station A510), respectively. All procedures involving animals were approved by the Ethics Committee on the Use of Production Animals of the Universidade Federal de Vicosa, MG - Brazil, under protocol number 52/2021.

Forty pregnant Nellore cows were used, including 16 nulliparous and 24 multiparous, averaging 429 ± 40.9 kg and 523 ± 24.0 kg of body weight, respectively, and body condition score (BCS) of 6.4 ± 0.3 and 6.3 ± 0.6 and age of 2.5 ± 0.5 y and 6.2 ± 2.3 y, respectively. Because evaluations were conducted during the postpartum period, nulliparous cows were thereafter referred to as primiparous cows. The experimental design was completely randomized, in a 2×2 factorial arrangement, where two parity orders were evaluated: primiparous (**P1**) and multiparous (**P2**), and two postpartum supplementation strategies, as follow: unsupplemented (**N**): cows receiving only mineral mixture *ad libitum*, and supplemented

(S): cows receiving 1.0 kg day⁻¹ of protein supplement and mineral mixture *ad libitum* (percentage composition of the mineral mixture [g kg⁻¹]: dicalcium phosphate 500, sodium chloride 471.9, zinc sulfate 15.0, copper sulfate 7.00, manganese sulfate 5.00, cobalt sulfate 0.50, potassium iodate 0.50 and sodium selenite 0.10). The protein supplement was formulated to contain 25% crude protein on a dry matter basis, with the following composition (gkg⁻¹): wheat bran 475, cornmeal 350, soybean meal 150, and urea:ammonium sulfate [9:1] 25). It was provided daily at 11 a.m. Furthermore, animals received ecto- and endoparasite control as needed. Maternal treatments started at parturition and extended until day 42 postpartum. For the evaluations, the cows were allocated so that each paddock contained only one parity order, with 4 paddocks destined for animals of each parity order.

The cows and their respective offspring were weighed, without fasting, on the 42nd day postpartum, to assess the average daily gain (**ADG**). Simultaneously, the cow body condition score (**BCS**) was also evaluated by three experienced assessors, and the average was calculated for each animal.

Pasture samples were collected every 28 days to quantify total dry matter (**TDM**) and potentially digestible dry matter (**pdDM**). The forage availability was assessed by using a metal square (0.5 × 0.5 m) at four randomly chosen points within each paddock. Simultaneously, forage samples were taken from each paddock by hand-plucked sampling in order to assess the chemical composition of consumed forage. All samples were weighed, dried at 55°C for

72 h, and ground using a Wiley[®] mill (model 3, Arthur H. Thomas, USA).

To estimate milk yield, machine milking was performed at 7 and 42 d postpartum. Cows were separated from their calves at 18h00, and on the following day at 06h00, 2 mL of oxytocin (10 IU mL⁻¹, Ocitovet[®], Brazil) were injected in the mammary vein, and milking was conducted immediately afterward. The exact interval between calf separation and milking was recorded, and the milk produced was proportionally adjusted to a 24-h production basis (Lopes et al., 2022). Milk yield was corrected to 4%-fat (**CMY4%**) according to the National Research Council (2001): $CMY4\% = (0.4 \times MY) + (15 \times F) \times (100 \times MY)^{-1}$, where CMY = corrected milk yield (kg.day⁻¹), MY = milk yield (kg), F = fat (%). Moreover, a 30 mL sample of milk from morning and afternoon milking was collected from each cow to evaluate milk composition.

Blood samples were collected at 07h00 on days 0, 7, 14, 28, and 42 postpartum by jugular venipuncture using vacuum tubes containing clot activator and gel separator (BD Vacutainer[®] SST[®] II Advance[®], Brazil) for serum collection. Serum was used to quantify serum urea nitrogen (SUN), total protein, albumin, globulin, creatinine, triglycerides, total cholesterol, high-density lipoprotein (HDL), non-esterified fatty acids (NEFA), β -hydroxybutyrate (β HB), insulin, insulin-like growth factor 1 (IGF-1), total triiodothyronine (T3), and total thyroxine (T4). Blood samples for plasma glucose determination were collected into tubes containing EDTA and sodium fluoride (BD Vacutainer[®] Fluoride/EDTA, Brazil). After collection, blood samples were centrifuged at 3,600 × g for 15 min, and serum and plasma were separated into microtubes and stored at -20°C until analysis.

Samples of forage and supplement were oven-dried at 55 °C and subsequently processed to pass through 1 mm and 2 mm sieves. Samples of forage (hand-plucked) were analyzed for contents of dry matter (DM, dried for 16 h at 105 °C, INCT method-CA G-003/1), ash (complete combustion at 550 °C, method M-001/2), neutral detergent fiber (NDF, method F-013/1), with corrections for ash (CIDN, M-002/2) and protein (PIDN, N-004/2), and N (Kjeldahl method, INCT-CA method N-001/2) were evaluated according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science INCT-CA (Detmann et al., 2021), using the samples processed at 1 mm. The content of indigestible neutral detergent fiber (iNDF) was estimated through a 288 h in situ incubation procedure using samples processed at 2 mm (Detmann et al., 2021). In the forage samples for availability (i.e., TDM and pdDM), the contents of DM, NDF and iNDF were quantified, according to the methods described above.

Potentially digestible DM (pdDM) was estimated according to Paulino et al. (2004), as follows: $\text{pdDM} = 0.98 \times (100 - \text{NDF}) + (\text{NDF} - \text{iNDF})$. Milk samples were analyzed regarding protein, fat, lactose, and total solids contents using infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil). Bioclin® kits (Belo Horizonte, Brazil) were used to measure plasma glucose concentrations (K82) and serum concentrations of urea (K056), total protein (K031), albumin (K040), total cholesterol (K083), triglycerides (K117), and HDL (K071). Serum concentrations of NEFA and beta-hydroxybutyrate (β HB) were measured using Randox® kits (A115 and RB1007, United

Kingdom). All the above analyses, except for insulin-like growth factor 1 (IGF-1), were performed on an automated biochemical analyzer (Mindray Co. Ltd., BS-200E, China). Serum IGF-1 concentrations were measured using Siemens® kits (Berlin, Germany) on an automated chemiluminescence analyzer.

Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) values were estimated according to Friedewald et al. (1972) using the equation: $\text{CT} = \text{HDL} + \text{LDL} + \text{VLDL}$, where CT means total cholesterol (%), HDL means high-density lipoprotein (%), LDL means low-density lipoprotein (%), and VLDL means very low-density lipoprotein (%). The VLDL value was calculated as one-fifth of the measured amount of triglycerides. Globulins were calculated by the difference between total proteins and albumin. Serum urea nitrogen (SUN) was estimated as 46.67% of the serum urea concentration.

The statistical model used was: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$, where, Y_{ijk} = observations of the dependent variable corresponding to parity order j , subjected to postpartum supplementation strategy i in paddock k , μ = general constant, α_i = fixed effect of the supplementation strategy, β_j = fixed effect of parity order (multiparous and primiparous), $(\alpha\beta)_{ij}$ = interaction between fixed effects, e_{ijk} = random error.

The metabolic variables were analyzed in repeated measures over time, considering the interaction between treatments and days of evaluation as a fixed effect. Significances were declared if $P \leq 0.05$, and trends were declared when $0.05 < P \leq 0.10$. The residues were verified for normality by the Shapiro-Wilk test and the homogeneity of variances by the Bartlett test. All procedures were performed in the R software, Agricolae package.

Results and Discussion

The chemical composition of the forage and supplement is presented in Table 1. Our main objective was to determine whether primiparous cows would exhibit a greater productive and metabolic response to supplementation than multiparous cows. However, our hypothesis was not supported by the present findings. No interaction ($P \geq 0.28$) between parity order and supplementation was observed for any performance variable (Table 2). Nevertheless, supplemented cows tended ($P < 0.09$) to exhibit greater ADG regardless of parity order. In this study, forage crude protein concentration averaged 99.5 g/kg DM. Previous studies conducted under tropical conditions have demonstrated that even high-quality tropical forages may not provide an adequate balance between energy and protein supply for grazing cattle

(Detmann et al., 2014; Reis et al., 2020). Under these conditions, protein supplementation may improve nutrient utilization efficiency and increase metabolizable nutrient supply to the animal, thereby enhancing productive performance. This interpretation is consistent with previous studies conducted with Nellore cows under tropical grazing conditions (Almeida et al., 2020; Moreno et al., 2023).

Similarly, Parallely supplementation increased blood IGF-1 concentrations in both multiparous and primiparous cows (Table 2; $P < 0.02$). Hormones such as insulin and IGF-1 are important activators of the mTOR signaling pathway, that serves as a critical signaling factor that promotes protein synthesis and inhibits protein degradation (Takahara et al., 2020). Indeed, circulating IGF-1 concentrations is positively associated to the body nitrogen accretion (Reis et al., 2020).

Table 1
Chemical composition of the forage and supplement (g kg⁻¹ dry matter)

Item	Supplement	Forage
Dry matter, as-fed	930	313±0.43
Organic matter	997	919±0.14
Crude protein	231	99.5±0.27
Neutral detergent fiber	175	608±0.35
iNDF	73	308±0.39

iNDF: indigestible neutral detergent fiber.

Table 2**Effects of parity and supplementation on the performance of cows and their offspring**

Item	Treatment ¹				SEM	P-value ²		
	UP1	SP1	UP2	SP2		S	P	P × S
ADG _{Cow}	0.27	0.29	0.33	0.41	0.008	0.08	0.01	0.28
Initial BCS	6.5	6.2	6.5	6.3	0.07	0.09	0.57	0.53
Final BCS	6.3	6.4	6.2	6.4	0.06	0.20	0.53	0.50
Calf weight ³	30	35	37	37	0.9	-	0.01	-
Calf weight ⁴	79	84	84	90	6.3	<0.01	<0.01	0.35
ADG _{calv}	1.17	1.17	1.13	1.26	0.008	0.06	0.03	0.52
CMY4, kg	6.6	5.5	9.1	10.8	0.60	0.40	<0.01	0.20
Fat, %	3.8	3.3	4.8	4.7	0.20	0.30	<0.01	0.38
Protein, %	3.4	3.3	3.4	3.3	0.08	0.02	0.45	0.71
Lactose, %	4.2	4.6	4.5	4.6	0.06	0.02	0.44	0.16
Total solids, %	12.5	12.4	13.8	13.7	0.21	0.64	<0.01	0.83

¹Unsupplemented primiparous (UP1), supplemented primiparous (SP1), unsupplemented multiparous (UP2), and unsupplemented multiparous (SP2). ²S: supplementation effect; P: parity effect; P × S: interaction effect between parity and supplementation. SEM: standard error of the mean; CMY4: 4% fat-corrected milk yield. ³Calf birth weight; ⁴Calf weight at 42 days of age; ADG: average daily gain; BCS: body condition score

Parity order affected cow ADG ($P < 0.02$), with a higher 0.085 kg/d for the multiparous cows (Table 2), consistent with the findings of Nishimura et al. (2023) in Nellore cows. However, gain rate (kg day⁻¹ per 100 kg BW) was similar across parity orders, averaging 0.07 kg/100 kg BW. Calf birth weight and ADG were also affected by parity order ($P < 0.02$), with greater values for offspring born to multiparous calves (Table 2). Additionally, multiparous cows had higher corrected milk yield and higher concentrations of fat and total solids in milk than primiparous cows ($P < 0.02$). The greater ADG in calves from multiparous cows was associated to higher milk and milk solids production in multiparous cows. There is a consensus in the literature that primiparous Nellore cows have lower milk yield compared

to multiparous cows (Lopes et al., 2022). Indeed, secretory tissue volume and rate of tissue growth are greater during the second gestation than during the first in dairy cattle (Knight & Wilde, 1993) and the mammary gland of multiparous cows is metabolically more active than of primiparous cows (Miller et al., 2006). These physiological differences may partially explain the greater milk production observed in multiparous cows in the present study.

On the other hand, supplementation increased calf body weight at the 42 days of age ($P < 0.02$), regardless of parity order (Table 2). Supplementation increased lactose ($P < 0.03$), possibly due to the greater supply of glucose and/or gluconeogenic precursors (e.g., amino acids). Thus, the better performance of calves born to supplemented

cows is associated with increased lactose yield in milk, as demonstrated by Almeida et al. (2020). In contrast, the BCS was not affected by supplementation or parity order ($P > 0.20$).

Previous studies have suggested that primiparous Nellore cows exhibit different metabolic patterns postpartum compared to multiparous cows (Ferreira et al., 2021). Therefore, our objective was to understand whether supplementation would induce similar metabolic changes in primiparous cows as in multiparous cows. Overall, no interaction between parity order and supplementation was observed for most metabolic variables ($P \geq 0.24$), except in the case of albumin levels ($P < 0.05$), with the lowest concentrations observed in primiparous non-supplemented cows (Table 3), possibly due to the greater nutritional requirement and lower protein input by diet in this treatment. Furthermore, there was a day effect ($P < 0.02$) for albumin, with a reduction in its concentrations throughout lactation. The decrease in albumin concentration throughout lactation is due to the demand for amino acids by the mammary gland during early lactation (Cunningham and Klein, 2013).

Supplemented animals had lower concentrations of total cholesterol and LDL ($P < 0.02$) compared to non-supplemented

animals. Although the greater concentrations of total cholesterol and LDL observed in non-supplemented cows may indicate intense mobilization of body reserves, no differences were detected in NEFA concentrations and BCS between treatments.

Multiparous cows exhibited greater globulin concentrations than primiparous cows ($P < 0.02$; Table 3). Globulins are not reliable indicators of protein metabolism, being more closely associated within inflammatory and immunity responses (Ferreira et al., 2021). The lower serum globulin concentrations in primiparous cows may indicate greater globulin use in colostrum or simply reflect a lower capacity to produce high-quality colostrum. In fact, it has been documented that colostrum from primiparous cows in taurine beef cows has lower IgG concentrations compared to multiparous cows, which is reflected in the calf's antibody concentration (Brereton et al., 2024). Furthermore, primiparous cows had lower milk production than multiparous cows, and this trend is also observed for colostrum. Collectively, these findings reinforce the importance of greater management attention to calves born from primiparous cows, particularly regarding adequate passive immunity transfer.

Table 3

Effects of parity and supplementation on metabolite and hormone concentrations

Item	Treatment ¹				SEM	P-value ²					
	UP1	SP1	UP2	SP2		S	D	S × D	P	P × S	P × D
Glucose, mg dL ⁻¹	69.9	60.4	69.3	70.4	4.60	0.25	<0.01	0.94	0.18	0.14	0.26
Triglycerides, mg dL ⁻¹	20.8	22.7	23.6	24.5	0.56	0.25	0.24	0.52	0.01	0.84	0.33
Total cholesterol, mg dL ⁻¹	139	129	143	126	3.18	<0.01	<0.01	0.71	0.70	0.21	0.23
NEFA, mmol L ⁻¹	57.3	51.7	58.2	47.0	2.63	0.02	<0.01	0.32	0.37	0.83	0.14
LDL, mg/dL ⁻¹	76.0	79.6	79.5	77.0	1.31	0.53	<0.01	0.29	0.37	0.66	0.51
HDL, mg/dL ⁻¹	220	244	222	249	10.8	<0.01	0.47	0.26	0.44	0.26	0.33
IGF-1, ng/mL ⁻¹	11.3	11.5	12.3	11.2	1.01	0.70	<0.01	0.38	0.82	0.32	0.31
SUN, mg/dL ⁻¹	6.28	6.18	6.59	6.60	0.15	0.73	<0.01	0.57	0.82	0.32	0.32
Total proteins g/dL ⁻¹	3.04b	3.13a	3.28a	3.17a	0.08	0.70	<0.01	0.77	<0.01	0.04	0.24
Albumin, g/dL ⁻¹	3.24	3.05	3.31	3.43	0.07	0.98	<0.01	0.64	0.02	0.14	0.32
Globulins, g/dL ⁻¹	4.15	4.55	4.73	4.91	0.11	0.25	0.24	0.52	0.01	0.84	0.33
VLDL	4.15	4.55	4.73	4.91	0.11	0.25	0.24	0.52	0.01	0.84	0.33

¹Unsupplemented primiparous (UP1), supplemented primiparous (SP1), unsupplemented multiparous (UP2), and unsupplemented multiparous (SP2). ²S: supplementation effect; D: sampling day effect; S × D: interaction effect between sampling day and supplementation; P: parity effect; P × S: interaction effect between parity and supplementation; P × D: interaction effect between parity and sampling day; SEM: standard error of the mean; βHB: beta-hydroxybutyrate*; NEFA: non-esterified fatty acids; IGF-1: insulin-like growth factor; SUN: serum urea nitrogen; LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very low-density lipoprotein;

Furthermore, there was an increase in blood globulin concentration throughout lactation ($P < 0.02$; Figure 1). In general, globulins are more closely related to the immunological memory of animals rather than to protein metabolism. Reduced globulin levels before parturition and at the beginning of lactation are explained by the transfer of immunity to colostrum production (Larson, 1958).

Multiparous animals exhibited greater concentrations ($P < 0.01$) of triglycerides and VLDL than primiparous cows (Table 3). Furthermore, there was an interaction effect between sampling day and parity

order ($P < 0.04$) for NEFA concentrations, with multiparous animals having greater concentrations than primiparous animals at parturition (Figure 3). Indeed, Cattaneo et al. (2023) found a greater apparent amount of adipose tissue mobilization in the second lactation compared to the first lactation in dairy cattle. Because milk yield is higher in multiparous cows, their nutrient demands during early lactation were likely greater than those of primiparous cows. Thus, they are more affected by the reduction in feed intake in the peripartum period. NEFA levels were similar between parity orders during postpartum period, which agrees with the similarity observed for BCS. Furthermore, the

greater NEFA concentrations at parturition in multiparous cows explain the higher blood concentrations of VLDL in this category. Very low-density lipoproteins play an important role in the transport of triglycerides synthesized in the liver to peripheral tissues, including mammary gland (Puppione, 1978), which also supports the greater fat yield of milk observed in multiparous cows.

There was an effect of sampling day ($P < 0.01$) for glucose and SUN. Indeed, previous studies with Nellore cows have consistently reported elevated glucose levels at calving, regardless of supplementation (Ferreira et al., 2020). This increase is associated with the hormonal response (e.g., cortisol and epinephrine) to the stress of calving. These hormones stimulate the release of amino acids and glycerol from muscle and adipose tissue, respectively, which in turn stimulates gluconeogenesis (Ralph & Tilbrook, 2016). This catabolic process may also explain the elevated blood urea nitrogen and NEFA levels observed at calving. Furthermore, near parturition, there is a decrease in feed intake in grazing Zebu cows, it seems to extend until the early days of lactation (David et al., 2024), potentially intensifying tissue mobilization during early lactation.

Sampling day affected total cholesterol, HDL and LDL concentrations ($P < 0.01$), with increases throughout lactation, which can be explained by the recovery of feed intake (David et al., 2024), as well as with the increasing metabolic demand for cholesterol during the re-establishment of reproductive activity (Holtenius et al., 2003). Cholesterol is the main precursor for steroid hormone synthesis, and HDL plays an important role in cholesterol transport to ovarian tissues. Indeed, granulosa cells primarily acquire

cholesterol through HDL uptake (Shalgi et al., 1973), which may partially explain the gradual increase in circulating HDL concentrations over time.

Conclusion

Postpartum supplementation improves the productive performance of cows under grazing, regardless of parity order. Overall, primiparous and multiparous cows exhibit similar metabolic adaptations during early lactation. However, multiparous cows have greater lipid mobilization around parturition. Unsupplemented primiparous cows exhibit the poorest protein metabolic status, indicating that this category may be more sensitive to inadequate nutrient supply during the postpartum period.

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References

- Almeida, D. M., Marcondes, M. I., Rennó, L. N., Martins, L. S., Marques, D. E. C., Saldarriaga, F. V., Villadiego, F. A. C., Ortega, R. M., Moreno, D. P. S., Moura, F. H., Cunha, C. S., & Paulino, M. F. (2020). Effects of pre- and postpartum supplementation on lactation and reproductive performance of grazing Nellore beef cows. *Animal Production Science*, 61(1), 101-107. doi: 10.1071/AN18251

- Brereton, N., McGee, M., Beltman, M., Byrne, C. J., Meredith, D., & Earley, B. (2024). Effect of suckler cow breed type and parity on the development of the cow-calf bond post-partum and calf passive immunity. *Irish Veterinary Journal*, 77(1), 1-13. doi: 10.1186/s13620-024-00276-x
- Cattaneo, L., Piccioli-Cappelli, F., Minuti, A., & Trevisi, E. (2023). Metabolic and physiological adaptations to first and second lactation in Holstein dairy cows: post prandial patterns. *Journal of Dairy Science*, 106(5), 3576-3585. doi: 10.3168/jds.2022-22685
- Coffey, M. P., Hickey, J., & Brotherstone, S. (2006). Genetic aspects of growth of Holstein-Friesian dairy cows from birth to maturity. *Journal of Dairy Science*, 89(1), 322-329. doi: 10.3168/jds.S0022-0302(06)72097-5
- Cunningham, J. G., & Klein, B. G. (2013). *Fisiologia veterinária* (5a ed.). Elsevier España.
- David, G. S. S., Matos, E. M. de A., Domingos, B. R., Ebani, Y. C., Sousa, L. C. O. de, Leite, G. D. O., Pereira, P. H. B., Rennó, L. N., Lopes, S. A., Valadares, S. de C., Fº., & Paulino, M. F. (2024). Periparturient changes in voluntary intake, digestibility, and performance of grazing Zebu beef cows with or without protein supplementation. *Animals*, 14(11), 1710. doi: 10.3390/ani14111710
- Detmann, E., Costa e Silva, L. F., Rocha, G. C., Palma, M. N. N., & Rodrigues, J. P. P. (2021). *Métodos para análise de alimentos* (2a ed.). Suprema.
- Detmann, E., Valente, E. E. L., Batista, E. D., & Huhtanen, P. (2014). An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. *Livestock Science*, 162, 141-153. doi: 10.1016/j.livsci.2014.01.029
- Ferreira, M. F. L., Rennó, L. N., Detmann, E., Paulino, M. F., Valadares Filho, S. C., Moreira, S. S., Martins, H. C., Oliveira, B. I. C., Marquez, J. A., & Cidrine, I. P. (2020). Performance, metabolic and hormonal responses of grazing Nellore cows to a nenergy-protein supplementation during the pre-partum phase. *BMC Veterinary Research*, 16(1), 108. doi: 10.1186/s12917-020-02309-3
- Ferreira, M. F. L., Rennó, L. N., Rodrigues, I. I., Detmann, E., Paulino, M. F., Valadares Filho, S. C., Martins, H. C., Moreira, S. S., & Lana, D. S. de. (2021). Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods. *BMC Veterinary Research*, 17(1), 311. doi: 10.1186/s12917-021-03019-0
- Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18(6), 499-502. doi: 10.1093/clinchem/18.6.499
- Holtenius, K., Agenäs, S., Delavaud, C., & Chilliard, Y. (2003). Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *Journal of Dairy Science*, 86(3), 883-891. doi: 10.3168/jds.S0022-0302(03)73671-6

- Knight, C. H., & Wilde, C. J. (1993). Mammary cell changes during pregnancy and lactation. *Livestock Production Science*, 35(1-3), 3-19. doi: 10.1016/0301-6226(93)90178-K
- Larson, B. L. (1958). Transfer of specific blood proteins to the lacteal secretions near parturition. *Journal of Dairy Science*, 41(8), 1033-1044. doi: 10.3168/jds.S0022-0302(58)91047-6
- Lopes, S. A., Lana Ferreira, M. F., Costa e Silva, L. F., Prados, L. F., Rodrigues, I. I., Rennó, L. N., Siqueira, G. R., & Valadares, S. C., F^o. (2022). Evaluation of nonlinear models to predict milk yield and composition of beef cows: a meta-analysis. *Animal Feed Science and Technology*, 294, 115455. doi: 10.1016/j.anifeedsci.2022.115455
- Miller, N., Delbecchi, L., Petitclerc, D., Wagner, G. F., Talbot, B. G., & Lacasse, P. (2006). Effect of stage of lactation and parity on mammary gland cell renewal. *Journal of Dairy Science*, 89(12), 4669-4677. doi: 10.3168/jds.S0022-0302(06)72517-6
- Moreno, D. S., Ortega, R. M., Paulino, M. F., Rennó, L. N., & Detmann, E. (2023). Pre- and postpartum supplementation strategies of performance and metabolic status of grazing beef cows. *Pesquisa Agropecuária Brasileira*, 58, e03102. doi: 10.1590/S1678-3921.pab2023.v58.03102
- Nasrollahi, S. M., Ghorbani, G. R., Zali, A., & Kahyani, A. (2017). Feeding behaviors, metabolism, and performance of primiparous and multiparous dairy cows fed high-concentrate diets. *Livestock Science*, 198, 115-119. doi: 10.1016/j.livsci.2017.02.017
- National Research Council. (2001). *Nutrient requirements of dairy cattle* (7th rev. ed.). National Academy Press.
- Neave, H. W., Lomb, J., Weary, D. M., LeBlanc, S. J., Huzzey, J. M., & von Keyserlingk, M. A. G. (2018). Behavioral changes before metritis diagnosis in dairy cows. *Journal of Dairy Science*, 101(5), 4388-4399. doi: 10.3168/jds.2017-13078
- Nishimura, T. K., Silva, A. G. da, Abitante, G., Dahlen, C. R., Goulart, R. S., Zamudio, G. D. R., Luz Silva, S., Santana, M. H. A., Saran, A., Netto, Leme, P. R., & Pugliesi, G. (2023). Effects of early weaning on the reproductive performance of suckled Nelore cows in the subsequent breeding season. *Journal of Animal Science*, 101(1), skad330. doi: 10.1093/jas/skad330
- Paulino, M. F., Figueiredo, D. M., Moraes, E. H. B. K., Porto, M. O., Sales, M. F. L., Acedo, T. S., Villela, S. D. J., & Valadares, S. C., F^o. (2004). Suplementação de bovinos em pastejo: uma visão sistêmica. *Anais do Simpósio Internacional sobre Produção de Gado de Corte*, Viçosa, MG, Brasil, 4.
- Puppione, D. (1978). Implications of unique features of blood lipid transport in the lactating cow. *Journal of Dairy Science*, 61(5), 651-659. doi: 10.3168/jds.S0022-0302(78)94424-7
- Ralph, C. R., & Tilbrook, A. J. (2016). Invited review: the usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science*, 94(2), 457-470. doi: 10.2527/jas.2015-9645
- Reis, W. L. S., Palma, M. N. N., Paulino, M. F., Rennó, L. N., & Detmann, E. (2020). Investigation on daily or every three

days supplementation with protein or protein and starch of cattle fed tropical forage. *Animal Feed Science and Technology*, 269, 114650. doi: 10.1016/j.anifeedsci.2020.114650

Shalgi, R., Kraicer, P., Rimon, A., Pinto, M., & Soferman, N. (1973). Proteins of human follicular fluid: the blood-follicle barrier. *Fertility and Sterility*, 24(6), 429-434. doi: 10.1016/S0015-0282(16)39730-8

Takahara, T., Amemiya, Y., Sugiyama, R., Maki, M., & Shibata, H. (2020). Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *Journal of Biomedical Science*, 27(1), 87. doi: 10.1186/s12929-020-00679-2

