

UNIVERSIDADE FEDERAL DE VIÇOSA

MATHEUS FELLIPE DE LANA FERREIRA

METABOLIC AND NUTRITIONAL CHARACTERISTICS OF BEEF COWS DURING PRE-CALVING AND LACTATION PERIODS

**VIÇOSA - MINAS GERAIS
2022**

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Luciana Navajas Rennó

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**METABOLIC AND NUTRITIONAL CHARACTERISTICS OF BEEF COWS DURING
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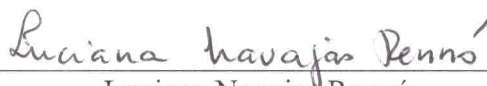
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Assent:



Matheus Fellipe de Lana Ferreira
Author



Luciana Navajas Rennó
Adviser

To my parents Willian and Roseli, my brothers Natália, Otávio and Caio, and my girlfriend Thalita for the encouragement, love, and unconditional support.

I dedicate.

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Thank you, so much!

*“The long and winding road
That leads to your door
Will never disappear
I’ve seen that road before
It always leads me here
Lead me to your door”
(Lennon/McCartney)*

BIOGRAPHY

Matheus Fellipe de Lana Ferreira, son of Willian José Ferreira and Roseli de Lana, was born in Viçosa, Minas Gerais, on November 24 of 1992.

He joined the Animal Science undergrad at Universidade Federal de Viçosa in 2011 and receive his bachelor's degree in 2016. In 2016, he started the Master's degree in the Department of Animal Science at the Universidade Federal de Viçosa, with major in Ruminant Physiology and Nutrition, concluding this course in March of 2018. Then, he started his PhD course in the same area and department, concluding the thesis in March of 2022, after a period of research scholar program in the University of Florida, from January of 2020 to August of 2020.

ABSTRACT

FERREIRA, Matheus Fellipe de Lana, D.Sc., Universidade Federal de Viçosa, March, 2022. **Metabolic and nutritional characteristics of beef cows during pre-calving and lactation periods.** Adviser: Luciana Navajas Rennó.

In this thesis, three chapters were prepared based on studies with Nellore cow-calf pairs. Two studies were developed in grazing conditions and a third study was developed in a feedlot facility. In the first chapter, the study aimed to evaluate how metabolic and hormonal traits change over time, their relationship to performance, and determine if these factors differ according to the parity order of grazing beef cows. Thirty-six pregnant Nellore cows (12 nulliparous, 12 primiparous, and 12 multiparous) were used. The study started at 60 d prepartum until 203 d of lactation. The initial body weight (BW) and final BW were higher for multiparous cows ($P > 0.05$). An interaction occurred between parity and day ($P < .0001$) for body condition score. Nulliparous and primiparous body condition score were reduced from -60 prepartum to 30 postpartum, then stabilized 60 postpartum ($P < 0.05$), while multiparous maintained body condition score from -60 days until 60 days postpartum ($P > 0.05$). Calf BW, final BW, and average daily gain did not differ between parities ($P > 0.05$). Effect of day ($P < 0.05$) was detected for non-esterified fatty acids, triglycerides, total cholesterol, LDL, VLDL, progesterone, and insulin. An interaction was observed between parity and days for glucose, HDL, β -hydroxybutyrate, creatinine and IGF-1 ($P < 0.05$). Parity affected serum urea nitrogen, total proteins, albumin, and globulins ($P < 0.05$). Parity and day relative to calving did not impact total T3 and T4 ($P > 0.05$). Hormonal and metabolic profile is strongly influenced by the stage of lactation. Time effects (pre-partum and lactation period) were more pronounced in nulliparous since they displayed more unbalanced metabolic and hormonal traits and lowered BCS pre- and postpartum. In the second chapter, the study aimed to evaluate the effect of parity order on milk yield (MY) and composition over time of grazing beef cows and to evaluate nonlinear models to describe the lactation curve. Thirty-six pregnant Nellore cows (12 nulliparous, 2 years; 12 primiparous, 3 years; and 12 multiparous, 4–6 years) were included in the study. Assigning calving day as day 0, milking was performed using a milking machine to estimate MY on days 7, 14, 21, 42, 63, 91, 119, 154, and 203. Dummy variable analyses were applied to estimate its effects on MY, composition (kg and percentage), afternoon/morning, and afternoon/total proportions. Since multiparous

had higher MY than nulliparous and primiparous, two different groups were used for lactation curve analysis: Mult (multiparous) and Null/Prim (nulliparous and primiparous). The MY estimated by the BR-Corte (2016) (Nutrient Requirements of Zebu and Crossbred Cattle) equation was compared with the observed values from this study. Five nonlinear models proposed by Wood (1967) (WD), Jenkins & Ferrell (1984) (JF), Wilmink (1987) (WK), Henriques et al., (2011) (HR) and Cobby & Le Du (1978) (CL) were evaluated. Models were validated using an independent dataset of multiparous and primiparous cows. The estimates for the parameters a, b, and c of the CL equation were compared between groups and the BR-Corte equation using model identity methodology. Nulliparous and primiparous displayed similar MY ($P>0.05$), however, multiparous cows had an average MY of 0.70 kg/d greater than nulliparous and primiparous ($P<0.05$). Milk protein and total solids were higher for multiparous cows ($P<0.05$). Effect of days in milking was found for milk fat, protein, and total solids ($P<0.05$). The yield of all milk components was higher for multiparous than nulliparous and primiparous. The afternoon/morning and afternoon/total proportion of milk production were not affected by parities and days in milking ($P>0.05$), with an average of 0.76 and 0.42, respectively. The BR-Corte equation did not estimate correctly MY ($P<0.05$). The equations of WD, WK, and CL had the best estimative of MY for both Mult and Null/Prim datasets. The equations had very similar Akaike's information criterion with correction and mean square error of prediction. In the third chapter, the study aimed to measure dry matter intake and digestibility of Nellore cows fed chopped sugarcane or *Brachiaria decumbens* grass in the feedlot during the peripartum. Ten pregnant multiparous Nellore cows, with average body weight (BW) of 500 ± 30 was used in this experiment. Cows were randomly divided into two treatments: *ad libitum* supply of sugarcane and *B. decumbens* grass. The sugarcane protein content was corrected with urea to reach the protein level of the *B. decumbens*. Cows received a 35% CP supplement during the pre-partum phase in the amount of 1 kg/animal/d and mineral mixed (MM) *ad libitum*. Cows were fed twice a day (7:00h and 15:00h) and intake was adjusted to allow minimal orts without DMI (dry matter intake) restriction. Intake was calculated by manually weighing the offered feeds and collected orts. The intake measurements started from 24 days before the expected calving date until 24 days postpartum. To evaluate intake and digestibility, a trial was run for 9 days on day 14 before the estimated parturition date and 14 days after parturition. The DMI was similar during pre-partum days except for the parturition that had a decrease of 30% of DMI ($P<0.05$). During pre-partum, the CP and digestible OM intake were higher ($P<0.05$) for the

sugarcane cows, but apNDF and iNDF intake were higher for *B. decumbens* cows ($P<0.05$). An interaction occurred between treatments and day for DMI, in which *B. decumbens* cows had higher DMI than sugarcane cows after 17 days post-partum ($P<0.05$). Effect of day was also found, where *B. decumbens* cows started to increase DMI after 17 days in kg per day and in % of BW. Total DM, OM, CP, apNDF, and iNDF intake were higher for *B. decumbens* cows during postpartum. Digestibility of DM, OM, and CP was higher for sugarcane cows ($P<0.05$) at the pre-partum and postpartum period. In summary, the DMI during pre-partum did no change regardless the roughage source, except for a severe decrease at parturition day. The DMI started to increase after 17 days post-partum for the *B. decumbens* treatment.

Keywords: *Bos indicus*. Gestation. Physiology. Nutrition

RESUMO

FERREIRA, Matheus Fellipe de Lana, D.Sc., Universidade Federal de Viçosa, março de 2022. **Metabolic and nutritional characteristics of beef cows during pre-calving and lactation periods.** Orientadora: Luciana Navajas Rennó.

Para esta tese, três capítulos foram elaborados com base em estudos com pares de vacas-bezerros da raça Nelore. Dois estudos foram desenvolvidos em condições de pastejo e um terceiro estudo foi desenvolvido em confinamento. No primeiro capítulo, o estudo teve como objetivo avaliar como as características metabólicas e hormonais mudam ao longo do tempo, sua relação com o desempenho e determinar se esses fatores diferem de acordo com a ordem de parto de vacas de corte em pastejo. Foram utilizadas 36 vacas Nelore prenhes (12 nulíparas, 12 primíparas e 12 múltiparas). O estudo começou aos 60 dias pré-parto até 203 dias de lactação. O peso corporal inicial (PC) e o PC final foram maiores para vacas múltiparas ($P > 0,05$). Ocorreu uma interação entre ordem de parto e dia ($P < .0001$) para escore de condição corporal. Nulíparas e primíparas reduziram o escore de condição corporal de -60 pré-parto para 30 pós-parto e estabilizaram em 60 pós-parto ($P < 0,05$), enquanto múltiparas mantiveram o escore de condição corporal de -60 dias até 60 dias pós-parto ($P > 0,05$). O PN das bezerras, o PN final e o ganho médio diário não diferiram entre as ordens de parto ($P > 0,05$). Efeito do dia ($P < 0,05$) foi detectado para ácidos graxos não esterificados, triglicerídeos, colesterol total, LDL, VLDL, progesterona e insulina. Foi observada interação entre ordem de parto e dias para glicose, HDL, β -hidroxibutirato, creatinina e IGF-1 ($P < 0,05$). A paridade afetou o nitrogênio uréico sérico, proteínas totais, albumina e globulinas ($P < 0,05$). A ordem de parto não influenciou as concentrações de T3 e T4 total ($P > 0,05$). O perfil hormonal e metabólico é fortemente influenciado pelo estágio de lactação. Os efeitos de tempo (pré-parto e período de lactação) foram mais pronunciados em nulíparas, uma vez que apresentaram características metabólicas e hormonais mais desequilibradas e reduziram o ECC pré e pós-parto. No segundo capítulo, o estudo teve como objetivo avaliar o efeito da ordem de parto na produção e composição do leite ao longo do tempo de vacas de corte em pastejo e avaliar modelos não lineares para descrever a curva de lactação. Trinta e seis vacas Nelore prenhes (12 nulíparas, 2 anos; 12 primíparas, 3 anos; e 12 múltiparas, 4-6 anos) foram incluídas no estudo. Atribuindo o dia do parto como dia 0, a ordenha foi realizada usando uma ordenhadeira mecânica para estimar o MY nos dias 7, 14, 21, 42, 63, 91, 119, 154 e 203.

Análises de variáveis dummy foram aplicadas para estimar seus efeitos sobre o MY, composição (kg e porcentagem), tarde/manhã e tarde/proporções total. Como as multíparas apresentaram maior MY do que as nulíparas e primíparas, dois grupos diferentes foram utilizados para análise da curva de lactação: Mult (multíparas) e Null/Prim (nulíparas e primíparas). O MY estimado pela equação BR-Corte (2016) (Requisitos Nutricionais de Bovinos Zebu e Mestiços) foi comparado com os valores observados neste estudo. Cinco modelos não lineares propostos por Wood (1967) (WD), Jenkins & Ferrell (1984) (JF), Wilmink (1987) (WK), Henriques et al., (2011) (HR) e Cobby & Le Du (1978) (CL) foram avaliados. Os modelos foram validados usando um conjunto de dados independente de vacas multíparas e primíparas. As estimativas para os parâmetros a, b e c da equação CL foram comparadas entre os grupos e a equação BR-Corte utilizando a metodologia de identidade de modelo. Nulíparas e primíparas apresentaram SM semelhante ($P>0,05$), porém, vacas multíparas apresentaram SM médio de 0,70 kg/d maior que nulíparas e primíparas ($P<0,05$). A proteína do leite e sólidos totais foram maiores para vacas multíparas ($P<0,05$). O efeito dos dias na ordenha foi encontrado para gordura do leite, proteína e sólidos totais ($P<0,05$). A produção de todos os componentes do leite foi maior para multíparas do que nulíparas e primíparas. A proporção tarde/manhã e tarde/total da produção de leite não foi afetada pelas paridades e dias de ordenha ($P>0,05$), com média de 0,76 e 0,42, respectivamente. A equação BR-Corte não estimou corretamente o MY ($P<0,05$). As equações de WD, WK e CL tiveram a melhor estimativa de MY para os conjuntos de dados Mult e Null/Prim. As equações tiveram o critério Akaike com correção muito semelhante e erro quadrático médio de predição. No terceiro capítulo, o estudo teve como objetivo mensurar o consumo de matéria seca e a digestibilidade de vacas Nelore alimentadas com cana picada ou *Brachiaria decumbens*. capim no confinamento durante o periparto. Dez vacas Nelore multíparas prenhas, com peso corporal médio (PV) de 500 ± 30 foram utilizadas neste experimento. As vacas foram divididas aleatoriamente em dois tratamentos: fornecimento ad libitum de cana-de-açúcar e *B. decumbens*. Relva. O teor de proteína da cana-de-açúcar foi corrigido com ureia para atingir o nível de proteína da *B. decumbens*. As vacas receberam suplemento de 35% de PB durante a fase pré-parto na quantidade de 1 kg/animal/de mistura mineral (MM) ad libitum. As vacas foram alimentadas duas vezes ao dia (7:00h e 15:00h) e a ingestão foi ajustada para permitir sobras mínimas sem restrição de CMS (ingestão de matéria seca). O consumo foi calculado pela pesagem manual das rações oferecidas e sobras coletadas. As medições de ingestão iniciaram a partir de 24 dias antes da data prevista para o parto até 24

dias pós-parto. Para avaliar o consumo e a digestibilidade, foi realizado um ensaio por 9 dias no dia 14 antes da data estimada do parto e 14 dias após o parto. O CMS foi semelhante durante os dias pré-parto, exceto para o parto que teve diminuição de 30% do CMS ($P < 0,05$). Durante o pré-parto, o consumo de PB e MO digestível foi maior ($P < 0,05$) para as vacas canavieiras, mas o consumo de FDNpp e FDNi foi maior para *B. decumbens* vacas ($P < 0,05$). Ocorreu uma interação entre tratamentos e dia para CMS, em que *B. decumbens* vacas apresentaram maior CMS do que vacas com cana-de-açúcar após 17 dias pós-parto ($P < 0,05$). Também foi encontrado efeito de dia, onde *B. decumbens* as vacas começaram a aumentar o CMS após 17 dias em kg por dia e em % de PV. O consumo total de MS, MO, PB, apFDN e FDNi foram maiores para *B. decumbens* vacas no pós-parto. A digestibilidade da MS, MO e PB foi maior para vacas canavieiras ($P < 0,05$) no pré-parto e pós-parto. Em resumo, o CMS durante o pré-parto não mudou independentemente da fonte de volumoso, exceto por uma diminuição severa no dia do parto. O CMS começou a aumentar após 17 dias pós-parto para a *B. decumbens* tratamento.

Palavras-chave: *Bos indicus*. Gestação. Fisiologia. Nutrição

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GENERAL INTRODUCTION

Shortening age at first calving is a strategy that maximizes the economic benefit of the production system as allows cows to have a longer lifetime productivity. However, primiparous cows that calve approximately at two years of age have often shown worse performance, lower pregnancy rate, and longer postpartum intervals in livestock systems than other categories (Triplett et al., 1995; Wiltbank, 1970). Studies with dairy cows showed that these animals are not physically or physiologically mature at this stage (Coffey et al., 2006) and there is still a lot to understand about the differences in metabolism according to parity (Wathes et al., 2007; Meikle et al. 2004).

The cow-calf operations are the base of the livestock system. Therefore, studies that provide a better understanding of the impact of the gestation and suckling period are important to optimize performance of the herd.

The study of the metabolic profile was developed by Payne & Payne (1970) but initially directed to clinical analyses. The use of metabolic profile analyses was further extended to relate nutrition and physiology, and to deep investigate impacts of a diet or supplementation on animal metabolism (Ferreira et al., 2020; Keogh et al., 2015 Yambayamba et al; 1996)

Such analyses of metabolites and hormones make possible to infer precisely about the animal metabolism, mainly for pregnant and lactating cows that are submitted to intense homeorhetic changes. The metabolic and hormonal outcome is basically a reflection of the animal performance. Comprehending and investigating those parameters during animal lifetime makes possible to apply a better nutritional management for both cow and calf pair.

The milking ability of beef cows is one of the main factors influencing calf preweaning growth and thus the weaning weight (Rutledge et al., 1971; Boggs et al., 1980). Considering that parity impacts the animal metabolism, it is likely that it would influence milk production and thus the performance of cow-calf pair. Besides the importance of milk yield measurement in studies with cow-calf pair, this evaluation enables the development of reliable equations to predict milk yield according to parity, which has not been taken into consideration yet on the Brazilian tables of Nutrient Requirements of Zebu and Crossbred Cattle (BR-Corte, 2016).

The accurate estimative of milk yield is essential as it shows the amount of nutrients being secreted into milk. This estimate is used to calculate the nutritional requirements of these animals (NASEM, 2016; BR-Corte, 2016). Therefore, it is also possible to identify

when the milk does not meet the nutritional requirements of the calves and the best time to introduce a creep-feeding supplementation to improve calves' performance.

Combining metabolic evaluations with thorough performance assessments, such as milk production and dry matter intake, we are able to fill lacks in knowledge and build bridges and paths that link phases of production and development whose investigation and understanding are essential for the prosperity and profitability of the livestock system.

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CHAPTER 1 - Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods - Article published at the BMC Veterinary Research (2021) - doi.org/10.1186/s12917-021-03019-0

Abstract

Background: Metabolic profile evaluation is a tool widely used in ruminant nutrition as metabolic cues that relate nutrition to physiology. Metabolic and hormonal traits change during pre-partum and lactation according to parity in dairy cows, but studies of beef cows under grazing are scarce. The present study aimed to evaluate how metabolic and hormonal traits change over time, their relationship to performance, and determine if these factors differ according to the parity order of grazing beef cows. Thirty-six pregnant Nellore cows (12 nulliparous, 12 primiparous, and 12 multiparous) were used. The study started at 60 d prepartum until 203 d of lactation.

Results: The initial body weight (BW) and final BW were higher for multiparous cows ($P > 0.05$). An interaction occurred between parity and day ($P < .0001$) for body condition score. Nulliparous and primiparous body condition score were reduced from -60 prepartum to 30 postpartum, then stabilized 60 postpartum ($P < 0.05$), while multiparous maintained body condition score from -60 days until 60 days postpartum ($P > 0.05$). Calf BW, final BW, and average daily gain did not differ between parities ($P > 0.05$). Effect of day ($P < 0.05$) was detected for non-esterified fatty acids, triglycerides, total cholesterol, LDL, VLDL, progesterone, and insulin. An interaction was observed between parity and days for glucose, HDL, β -hydroxybutyrate, creatinine and IGF-1 ($P < 0.05$). Parity affected serum urea nitrogen, total proteins, albumin, and globulins ($P < 0.05$). Parity and day relative to calving did not impact total T3 and T4 ($P > 0.05$).

Conclusions: Hormonal and metabolic profile is strongly influenced by the stage of lactation. Time effects (pre-partum and lactation period) were more pronounced in nulliparous since they displayed more unbalanced metabolic and hormonal traits and lowered BCS pre- and postpartum.

Keywords: blood total protein, *Bos indicus*, IGF-1, gestation, physiology

Background

Lifetime productivity of beef cows is affected by age at first calving. Beef cows are expected to begin breeding at approximately 13 to 14 months of age and calve for the first time at approximately 24 months of age as this maximizes the economic benefit of the production system [1]. However, animals are not physically or physiologically mature at this stage. So, cows experiencing their first calving are therefore in a different metabolic state than multiparous cows [2] as they require nutrients for their continued growth and the development of their calf.

In dairy cows, parity can influence the pattern of changes in metabolic hormones and metabolites following calving. However, even in dairy research in which the majority of ruminant metabolic profile studies have been conducted, published data are inconsistent [3–5].

Metabolic profile evaluation is a tool widely used in ruminant nutrition as metabolic cues that relate nutrition to physiology [6]. It helps to accurately indicate the effects of a diet [7,8] or supplementation on animal metabolism, as well as understand homeorhetic states such as gestation and lactation [9–11] in which changes in metabolism occur to establish a new physiological state.

The metabolic processes that communicate the nutritional status of the animal are complex and result in changes in several metabolites and hormones. Non-esterified fatty acids (NEFA) and β -Hydroxybutyrate (β HB) concentrations are an index of lipid mobilization and fatty acid oxidation, so high concentrations suggest an energy deficit; while blood total protein and albumin concentrations are used as strong indicators of protein metabolism [6]. Moreover, hormones as insulin-like growth factor-I (IGF-I) and insulin are linked to both energetic and protein status [12], hence, highly associated with milk production [13] and reproduction [14]. Among the metabolic traits usually assessed, IGF-1, β HB, and NEFA concentrations are the main physiological parameters reported to be inconsistent between parity orders studies [3,5].

While most of the scientific information regarding metabolic changes during transition period and lactation has been generated in confined systems, studies of range cattle under grazing and tropical conditions are scarce.

We hypothesized that parity influences metabolic and hormonal profile in beef cows under grazing where less mature cows display worst performance and more unbalanced metabolic traits. Therefore, the present study aimed to evaluate how metabolic and hormonal

traits change over time, their relationship to performance, and determine if these factors differ according to the parity order of grazing beef cows.

Results

The initial body weight (iBW) and final body weight (fBW) were higher for multiparous cows ($P > 0.05$). All cows average daily gain (ADG) measurements were similar between parities ($P > 0.05$). Calf iBW, fBW, and ADGs did not differ between parities ($P > 0.05$; Table 2).

An interaction occurred between parity and days relative to calving ($P < .0001$) for body condition score (BCS). Nulliparous and primiparous BCS were reduced from -60 prepartum to 30 postpartum, then stabilized 60 postpartum ($P < 0.05$), while multiparous maintained BCS from -60 days until 60 days postpartum ($P > 0.05$; Figure 1).

Milk yield (MY) was higher for multiparous ($P < 0.05$), and nulliparous and primiparous displayed similar MY ($P > 0.05$). Effect of days in milking was also detected for MY, protein and total solids ($P < 0.05$; Table 3). Milk yield of all parities was lowered at day 119 ($P < 0.05$).

An interaction occurred between parity and days relative to calving for glucose concentrations ($P < 0.001$; Table 4). Concentrations were higher for the nulliparous upon calving ($P < 0.001$; Figure 2a).

Effect of day ($P < 0.0001$; Table 4), but not parity or parity and day, were detected for triglycerides, total cholesterol, LDL and VLDL ($P > 0.05$). Triglyceride decreased after calving and then remained stable up to 21 days, where it started to increase. Highest concentrations were observed at 154 days postpartum and abruptly decreased at d 203 ($P < 0.05$; Figure 2b). Cholesterol and LDL decreased up to calving and then increased, achieving the highest values at d 203 ($P < 0.05$; Figure 2c and Figure 2d). VLDL concentrations followed the same pattern of triglyceride (Figure 2f).

Effects of parity and days relative to calving were detected for HDL: concentrations were higher for multiparous cows at day -21 and lower for nulliparous at day 21 ($P < 0.0001$; Figure 2d).

Effect of day ($P < 0.0001$; Table 4), but not parity or parity and day, were detected for NEFA. The NEFA serum concentrations had lower concentrations pre-partum at day -7, then peaked at calving, lowered at 7, 14, and 21 and stabilized after day 42 ($P < 0.05$; Figure 3a).

An interaction was observed between parity and days relative to calving for β HB ($P < 0.020$; Table 4), with higher concentrations on -14 and -7 days for nulliparous, higher for multiparous and primiparous than nulliparous on day 7, and higher for primiparous than the other categories at day 21 (Figure 3b).

Parity affected SUN, total proteins, albumin, and globulins (Table 4). Total protein increased with parity, and concentrations were highest for multiparous, followed by primiparous and nulliparous ($P = 0.002$; Figure 4a). Globulin concentrations were higher for multiparous and primiparous than nulliparous ($P = 0.021$; Figure 4c). Albumin was higher for multiparous than nulliparous and primiparous (Figure 4b), whereas SUN was lower for multiparous ($P = 0.023$; Figure 5a).

An interaction occurred between parity and days relative to calving for creatinine ($P = 0.023$) and IGF-1 ($P = 0.010$; Table 4) concentrations. For creatinine, concentrations were lower for the nulliparous on day 203 ($P < 0.05$; Figure 5b). For IGF-1, concentrations were lower for nulliparous cows at days 7, 14, and 21 and higher for multiparous than nulliparous at days 42, 63, and 91 ($P = 0.02$; Figure 6a).

Effect of days relative to calving ($P < 0.0001$; Table 4) were detected for insulin and progesterone, where insulin concentrations peaked on day -7, decreased until day 42 and then increased again, surpassing the first peak ($P < 0.05$; Figure 6b). Progesterone levels were higher on days 91 and 119 ($P < 0.0001$; Figure 6c).

Parity and days relative to calving did not impact total T3 and T4 concentrations ($P > 0.05$; Table 4).

Discussion

Concentrations of glucose were similar throughout the study and maintained at a basal level, although they reached high concentrations upon calving. A high blood glucose variance is not expected since homeostatic mechanisms control blood glucose concentration [15]. However, during parturition, cortisol and epinephrine levels increase, leading to glycogenolysis [16] and thus increased blood glucose [9,17]. In this study, nulliparous cows displayed higher glucose concentrations at calving, possibly due to more excitable temperament, stress, and fear experienced during calving than the other parities [18]. Nevertheless, cows had higher cortisol concentrations upon calving than the other days evaluated but did not show a parity effect (data not shown). Unfortunately, more

physiological stress parameters are needed to infer about parity effect on stress at calving accurately, which is not our goal in this study.

NEFA concentrations peaked upon calving, and values were maintained at basal level after 42 d post-calving. Similar to glucose, heightened NEFA at parturition is likely due to the catabolic effect of stress hormones, plus dry matter intake reduction, which elicits fatty acid mobilization. It appears that hormones involved in promoting energy mobilization during stress are synergic. In adrenalectomized animals, the lipolytic response to epinephrine is reduced, indicating that glucocorticoids at least facilitate epinephrine-stimulated lipolysis. So, both cortisol and epinephrine, which are released during stress, can also influence lipid and muscle metabolism [16,19]. Previous experiments with Nellore cows have often shown high NEFA and glucose upon calving regardless of supplementation levels [9,17]. Besides acting as a potent lipolytic factor, epinephrine stimulates muscular glycogenolysis and amino acid output [20], which would also explain high levels of blood urea upon calving. Amino acids can be used as a gluconeogenesis source; the deamination process releases their amine groups, which will then increase and be converted to urea by the liver [15]. Moreover, muscular glycogenolysis and lipolysis may contribute to enhanced hepatic gluconeogenesis and posterior heightened blood glucose by providing lactate and glycerol, respectively, as additional substrates to the liver [15]. Parturition is indeed an intense event in which physiological changes and hormonal interactions occur that can lead to data misinterpretation in experimental conditions; so, it is worth discussing the interrelationship between metabolites and hormones during this period.

Cholesterol levels progressively increased on all postpartum days regardless of the category, a response to an increase in dry matter intake and homeorhetic changes of lactation [21]. Cholesterol levels also follow this pattern regardless of the nutritional plan in beef cows [9,22] due to the higher need for lipoproteins to carry triglycerides to the mammary gland. Especially during this period, HDL concentrations are higher than the other lipoproteins, possibly due to either increased synthesis or VLDL catabolism by mammary tissue [23]. Therefore, the lower concentrations of triglycerides in the first weeks after calving is a combination of its utilization as energy for lactation as sources of fatty acids for milk fat synthesis [24] and depressed dry matter intake (DMI).

Increased cholesterol during postpartum could also be related to precursors being needed to synthesize steroidal hormones [21,25]. HDL appears to be more important during this period since it participates directly in reestablishing reproductive activity. While

reproductive activity is reestablished, avascularized granulosa cells are restricted to HDL cholesterol uptake [26]. Although progesterone did not differ between parities, reduced HDL concentrations of nulliparous cows at 21 days postpartum compared to the other categories could be possibly related to a delayed reestablishment of the reproductive activity. Besides HDL, differences in energy metabolism were also found for β HB.

Previous dairy cow studies have often shown higher NEFA and β HB for multiparous cows during lactation [5,27], and they associated it with higher milk yields of this category, due to fat mobilization for milk production. However, in the current experiment, multiparous cows displayed higher β HB only during the first week postpartum, and no parity effect on NEFA concentrations. For *Bos taurus* beef cows, Sinclair et al. [10] found higher β HB postpartum for primiparous than nulliparous during the first postpartum weeks, consistent with increased β HB for primiparous at days 7 and 21. Based on the contrasting results of NEFA and β HB between studies, it seems that energy status parameters are impacted differently between parity in dairy and beef cows since they differ in milk production potential.

The BCS loss at this time explains high levels of β HB for nulliparous cows pre-calving. They suggest that this category were in a worse energy status before calving [3], which is likely to be due to higher nutritional requirements than other categories, leading to more intense energy mobilization. Nulliparous cows normally start late gestation with a good BCS since they have not been previously challenged by a gestation plus lactation. Compared to the other categories, they could not maintain BCS in the late gestation and early lactation period, even though supplementation was provided during pre-partum. Although the primary objective of this experiment was to study the metabolic and hormonal changes rather than dam reproductive efficiency, it is important to emphasize that nulliparous (primiparous after calving) have often shown lower pregnancy rates and longer postpartum intervals in livestock systems than multiparous [28–30]. Although metabolic signals mediating reproduction are not fully understood, high β HB is known to be responsible for impaired reproduction [31]. The nutritional status upon calving is the main factor influencing the length between calving and conception [32]. Elevated levels of β HB concentration pre-calving are correlated to BCS loss, and thus to delayed luteal activity [5,33]. The levels of β HB of all parities are within the normal range for beef cows [9,34] and do not suggest a very severe energy deficit. Rather than that, it shows that nulliparous had worst energy balance pre-calving compared to other parities, which could impact future reproduction performance.

However, ADG prepartum was similar between parities because the nulliparous is still in continuous growth, so muscle and skeletal gain might have balanced out the fat mobilization. Therefore, it was expected that the pattern of protein metabolism changes would be more intense than the energy metabolism parameters between parities.

Similarly, as presented here, studies presenting parity comparisons in dairy cows also showed increased blood total proteins with increasing parity and following the same trend with respect to the days relative to calving [35]. Total protein and albumin parameters are long-term indicators of protein metabolism [36]; thus, reduced concentrations for the less mature animals could be related to reduced protein intake. However, in this case, it is more likely due to the deviation of amino acids from albumin synthesis to other body tissues as a homeorhetic mechanism since these categories require nutrients for fetus development, lactation, and continued growth.

Multiparous cow's albumin concentrations remained most of the time within the physiological range, but concentrations of albumin during lactation for primiparous and nulliparous were above the range (3.03-3.55 g/dL) [37]. Pronounced drop in plasma albumin above the reference values for primiparous and nulliparous may reflect a severe protein deficit [6]. On the other hand, González et al. [34] found for beef cows an overall average of plasma albumin concentration of 3.33 ± 0.407 g/dL, with extreme values between 2.18 to 3.78 g/dL.

Furthermore, it could also be assumed that the differences in blood total protein are due to different immunoglobulin concentrations between parities. The parity order impacted globulin concentrations similar to that described in several dairy cow studies: higher for multiparous and primiparous than nulliparous [35,38]. These differences are likely due to the more mature immunological memory of older animals, (i.e., likely higher antibody titers against a broader spectrum of antigens). Globulins are not good indicators of protein metabolism and are more important as indicators of inflammatory responses and immunity. The pattern of changes in globulin concentrations during pre- and postpartum is well established in dairy cows. Authors have suggested that enhanced globulins with age are explained by a specific increase in IgG1 and IgG2, with IgG1 tending to level off and IgG2 continuing to increase, while serum IgM and IgA concentrations show no age-response [39–41]. Reduced globulin levels before calving are justified by the transfer of immunity to colostrum production [40]; so, after calving, it increases linearly, corroborating previous studies from our group [9,17]. Despite being lower for primiparous and nulliparous, globulin

values found in this study corroborates with reference limits for Holstein cows (2.5 to 6.6 g/dL) [35,38].

To our knowledge, no studies have evaluated extensively the metabolic and hormonal traits in beef cows of different parity; therefore, most of the data herein cited for comparisons originated in studies of dairy cows.

Blood urea is considered a short-term protein indicator, and unlike the other indicators of protein status, SUN levels were lower for multiparous cows. SUN is often related to DMI and protein intake. In this case, it is very unlikely a reduced DMI for multiparous compared to the other categories since all cows were provided with the same pasture conditions and supplementation.

However, although cows had a good quantity and quality of forage available, a more intense DMI reduction postpartum may have limited energy intake for nulliparous and primiparous. If dietary energy supply is restricted, the rate of ammonia production from dietary CP exceeds the ability of the microbiota to convert it into microbial protein (lack of carbon skeleton), hence circulating ammonia concentrations will rise and be converted to urea by the liver [42]. Increased SUN can also be related to the mobilization of amino acids for gluconeogenesis, so the deamination process is responsible for the enhanced blood urea (a by-product of protein catabolism). For young cows, Sinclair et al. [10] suggested a preference for catabolism of lean tissue rather than fat tissue during the early postpartum period, although some studies did not find a difference in SUN between parities [38,43]. Also, a likely explanation that supports these outcomes is that as multiparous cows calved with better BCS, they had more adipose tissue to support milk production, so they had to mobilize less protein to support gluconeogenesis. Therefore, these urea metabolism differences are inherent to the categories' physiological state and the result of a possible combination of amino acid output and limited energy intake supply.

Those explanations are also supported by the lower creatinine values for nulliparous cows at the end of the study (d 203), suggesting that this category could not nutritionally overcome lactation, leading to lean tissue mobilization, which impacted muscular mass. Creatinine concentration is reported to be an index of muscle mass [44], and values slightly higher (although not significant) for nulliparous at -21 d have been reported for dairy cows [35]. These values are explained by the higher relative muscular mass of heifers. Creatinine concentrations of all categories began to recover after 63 d postpartum. Its decreasing concentrations after parturition are due to body weight loss [9], consistent with the negative

ADG postpartum until 60 d. Although changed throughout pre-partum and lactation, creatinine concentration is within the physiological range (1–2 mg/dL) [37].

These differences in energy and protein metabolism led to significantly lower milk production in first calving cows, as the differing metabolic traits may limit the partitioning of nutrients into milk. However, even receiving less milk, calves born from nulliparous cows had similar performances in comparison with other categories.

Explaining the weight gain in the calf by the cow's milk production can be quite complex, as calves tend to consume similar quantity of metabolizable energy per unit of weight [45], which means that, if milk consumption is reduced, calf increases forage intake in attempt to meet nutritional requirements [46]. Indeed, an upcoming study (with the same animals) from our group reveals that calves born from nulliparous cows had more grazing time than calves born from older cows (Rodrigues et al., unpublished data), possibly in response to less milk ingestion, which contributed to similar ADG between the calves.

There are no conclusive results in the literature regarding the IGF-1 concentration with respect to parity. While some studies have shown higher IGF-1 for nulliparous than multiparous cows [5,43], we found lower IGF-1 concentrations for nulliparous in the early lactation than the other categories, and values remained lower than multiparous cows until 119 days, which is consistent with Meikle et al. [3]. Even though these categories are in a different physiological state, IGF-1 concentrations during postpartum are more likely to be physiologically linked to milk production and nutritional status than growth. These results, combined with the lower BCS postpartum for nulliparous cows, reinforce that this category struggled to cope with lactation requirements. IGF-1 has an essential role in the galactopoiesis and persistency of lactation by decreasing the loss of secretory cells during lactation and by increasing cell proliferation [13,47], which corroborates higher milk production observed in multiparous cows [4,38]. Moreover, IGF-1 is also a good indicator of the capacity to resume reproductive activity after parturition. Lower concentrations of IGF-1 for nulliparous cows during this period explains the longer postpartum interval for this category than other categories widely shown in the literature [28–30].

Many variables influencing IGF-1 concentrations can explain differences between studies, such as production system (energy intake) and genetic background. Circulating IGF-1 is synthesized mainly in the liver, where its production is stimulated by the action of GH on GH receptors (GHR). Decreasing serum glucose concentrations and, consequently, insulin leads to a reduction in the GHR in the liver, the main mediator of IGF-1 production [12].

Thus, the contrasting results between dairy and beef cows could also be related to the different regulation of the GH receptor in the liver. It has been shown that the liver GHR (GHR1A) is downregulated during the parturition period in dairy cows but not in beef cows [48,49].

Both IGF-1 and insulin concentrations peaked at calving day due to increased glucose [9]. A linear increase of insulin concentrations after 42 days postpartum is related to a recovery of the DMI leading to better energy balance. Similar to insulin, both total T3 and T4 are strongly related to DMI and energy nutritional status, successfully responding to changes in beef cattle diet [7,9,50]. Their concentrations decrease due to energy mobilization status, slowing down the basal metabolism to lower maintenance requirements. These categories are indeed in different physiological states; therefore, the lack of differences between parities for T3 and T4 is unexpected as they are mainly responsible for basal metabolism and growth. A likely explanation is that T3 and T4 are more sensitive to changes in energy metabolism [50], while, in this study, as shown above, protein metabolism was more impacted by parity.

Thyroid hormones are galactopoietic and may play an important role in the regulation of lactation. Nevertheless, we found no significant differences in T3 and T4 concentrations during pre-partum or lactation period. Thyroid hormone concentrations throughout lactation have been found to vary in different studies. Some found no differences in T4 concentrations throughout lactation [51], while others reported that serum T4 concentrations were lower in early than in later lactation [52,53].

Although being extremely helpful to understand changes in nutrition, there are still conflicting results about thyroid hormones regarding homeorhetic changes in metabolism of dairy and beef cows. Based on these evidences, more studies are needed to elucidate how these hormones change according to gestation and lactation in *Bos indicus* beef cows.

In summary, these underlying changes in the physiology of nutrient balance and utilization are strongly influenced by the stage of lactation. These outcomes suggest that beef cows, regardless of their parity, begin to recover their nutritional status after 42 to 63 days postpartum, based on the negative ADG until 60 d and the return of the majority of hormones and metabolites to a normal level at this time. Notably, the recovery of the nutritional status also matches the higher levels of progesterone, which is physiologically consistent, due to the reestablishment of the reproductive activity.

Late gestation and lactation homeorhetic changes affected the metabolism of the categories at different magnitudes. Although there were some differences in energy

metabolism, these results suggest that the different metabolic and endocrine support between parities is more pronounced in protein metabolism. Because despite both urea and IGF-1 are also responses to the energy status, parity directly influenced all of the protein status indicators (i.e., total protein, albumin, globulins, urea, and IGF-1). Furthermore, nulliparous were more impacted by the pre-partum and lactation periods since they displayed more unbalanced metabolic and hormonal traits and lowered BCS pre- and postpartum.

Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 120/2018). Animals used in this study were provided by the Animal Science Department's beef cattle farm at the Universidade Federal de Viçosa, Viçosa-MG, Brazil, where the study was conducted from July 2018 to May 2019.

Experimental design and animals

Thirty-six pregnant Nellore cows (12 nulliparous, 12 primiparous, and 12 multiparous) were included to the study, with the following average age, BW, and BCS: 2 years, 442± 62 kg, 6.20± 0.5; 3 years, 457± 58 kg, 5.68± 0.5; 4-6 years, 505± 60 kg, 5.92± 0.5, respectively. The study started at 60 d prepartum until 203 d of lactation (two weeks before weaning). The nomenclature for each category related to parity was set at the beginning of the experiment and used throughout the manuscript, even though after calving, the parity order changed (e.g., nulliparous became primiparous cows).

Animals were randomly divided into six paddocks, and 2 females from each category were introduced into the paddocks 15 days before the beginning of the experiment to acclimate to the environment and the herd. The average area of the paddocks was 7 hectares, evenly covered with *Urochloa decumbens* grass, and cows were provided free access to water and feeders.

All cows were group-fed with an energy-protein supplement (1.0 kg/d) with 35% crude protein (CP) for 60 days prepartum (Table 1). The supplement was calculated to supply approximately 40% of cow's protein requirements, as recommended by BR-CORTE [54]. We provided a linear trough space of 0.70 m per cow to ensure homogeneous supplement intake among animals. The supplement was supplied at 1200 h.

After calving, cows remained at the same paddocks, and a commercial mineral mix (CaHPO₄ = 50.00%; NaCl = 47.775%; ZnSO₄ = 1.4%; Cu₂SO₄ = 0.70 %; CoSO₄ = 0.05%; KIO₃ = 0.05% and MnSO₄ = 0.025%) was also offered to cow-calf pairs for *ad libitum* intake throughout the experiment, supplied separately in additional feeders. Calves were offered 5 g/kg BW of an energy-protein supplement formulated to contain 20% CP in a creep-feeding system from 90 days of age until the end of the study (d 203).

During breeding season, which started around 70 days after parturition, cows were synchronized, and fixed-time artificial insemination was performed, as a usual annual procedure of the beef cattle farm sector.

Data collection

Cows were weighed at the beginning of the experiment (iBW; 60 d prepartum) and 7 d before the expected calving day to quantify the average daily weight gain pre-calving (ADGpre). Moreover, cows were weighed after calving, before the beginning of the breeding season (d 60) to quantify the average daily gain post-calving (ADGpost), and at the end of the experiment (fBW; d 203) to quantify the average final daily gain (ADGf). The BCS was also recorded on a scale from 1 to 9 [55], by three experienced evaluators at the beginning of the experiment (iBCS; -60 d), -30 d, 30 and 60 d postpartum.

Calves were weighed immediately after birth and on 2 consecutive days to determine both full and shrunk BW (14 hours) at the beginning of the creep-feeding phase (d 90) and the end of the experiment (d 203). Birth weight and full BW were used to determine ADG before the creep-feeding phase (ADGpr). Shrunk BW was used to determine calves ADG from the beginning of creep-feeding to the end of the experiment (ADGpo).

Forage sampling

Every 30 d, grass samples were collected using two methods: hand plucking to evaluate the forage selected by animals and cutting at the ground level from five delimited areas (0.5 x 0.5 m), selected randomly in each paddock to quantify total dry matter (DM) per ha. All samples were weighed, oven-dried (55°C), then ground to pass through 1- and 2-mm screens in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA). All data from each month were combined and expressed as an average per season as follows: dry season = July and August (beginning of the experiment), dry-to-rainy transition season = September to

November; rainy season = December to February; rainy-to-dry transition season = March to May (end of the experiment).

The average DM availability of forage was: dry season = 4.69 t/ha, dry-rainy transition = 4.33 t/ha; rainy season = 2.93 t/ha; rainy-dry transition = 3.74 t/ha. Supplement chemical composition and forage chemical composition according to the season are presented in Table 1.

Blood sample collection

Assigning calving day as day 0, blood samples were collected from cows before feeding on days -21, -14, -7, 0, 7, 14, 21, 42, 63, 91, 154, 119, and 203. Samples were collected by jugular vein puncture, using vacuum tubes with a clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) to quantify serum urea nitrogen (SUN), total protein, albumin, creatinine, triglycerides, total cholesterol, high-density lipoprotein (HDL), NEFA, β HB, insulin, insulin-like growth factor (IGF-1), total triiodothyronine (T3), total thyroxine (T4), and progesterone. A tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantify the plasma glucose concentration. After collection, samples were centrifuged at $2200 \times g$ for 20 min. Serum and plasma were immediately frozen at -20°C until analyzed.

Milk sampling

Milking was performed using a milking machine to estimate MY on days 21, 42, 63 and 119 of lactation. Milking procedures were made as described by Boggs et al. [56] which has controlled suckling period before the calf separation. To empty udders, calves were separated from their mothers from 3:00 pm to 5:45 pm, when they were reunited to dams and allowed to suckle. At 18:00 pm, calves were once again separated from dams until the next morning. At 06:00 am on the next day, cows were milked immediately after an injection of 10 UI of oxytocin (10 UI/mL; Ocitovet®, Brazil) in the mammary vein, and the produced milk was weighed. The exact time when the milking of each cow ended was recorded. Calves were kept away from their mothers until the next milking at 06:00 pm to obtain a 24-hour milk production. Then, the total milk yield was calculated as the sum of both milkings. Moreover, 30 mL sample of milk from morning and afternoon milking was collected from each cow to evaluate milk composition.

Analyses of feed, metabolites, hormones and milk

The forage and supplement samples grounded to pass through 1 mm screen were analyzed following the procedures described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA) [57] for DM (method G-003/1), ash (method M-001/1), CP (method N-001/1), and neutral detergent fiber corrected for ash and protein (aNDF; method F-002/1). Indigestible neutral detergent fiber (iNDF) [58] was quantified in samples to pass through 2 mm using *in situ* incubation procedures with nonwoven textile bags (100 g/m²) for 288 h.

Blood concentrations of urea (K056), total protein (K031), albumin (K031), triglycerides (K117), total cholesterol (K083), HDL (K071), and glucose (K082) were determined using Bioclin® kits (Belo Horizonte, Brazil). NEFA and β HB were analyzed using Randox® kits (FA115 and RB1007, Antrim, UK). All the above-mentioned analyses were determined by chemiluminescence method in an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). Insulin, total T3, total T4, and progesterone were analyzed by Beckman Coulter® kits (33410, 33830, 33800, and 33550, Brea, USA). IGF-1 contents were quantified using Siemens® kits (Berlin, Germany) in an automated chemiluminescence analyzer (Berlin, Germany). The intra- and inter-assay CV were, respectively, 2.3 and 4.5% for insulin, 4.1 and 5.9% for T3, 3.7 and 4.8% for T4, 3.8 and 5.6% for IGF-I, and 6.8 and 8.1% for progesterone.

The serum contents of low-density lipoprotein (LDL) and very-low density lipoprotein (VLDL) were calculated by the equation: $TC = HDL + LDL + VLDL$, where TC = total cholesterol and $VLDL = \text{triglycerides}/5$ [59]. Globulins were calculated by the difference between total proteins and albumin. SUN was estimated as 46.67% of total serum urea.

Milk samples were analyzed regarding protein, fat, lactose, and total solids contents using infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil).

Statistical Analyses

The basic statistical model was used as follow:

$$Y_{ijk} = \mu + P_i + C_j + e_{(ij)k}$$

where: Y_{ijk} = observation taken on animal k, pertaining to parity j, within paddock i; μ = overall constant; P_i = paddock effect I (random); C_j = Category (parity) effect j (fixed) $e_{(ij)k}$ = random effect, unobservable, assumed to be NIID (0, σ^2_e);

Blood parameters, milk yield and BCS taken over time in the same animals were evaluated as repeated measurements, where the best structure of (co)variance matrix was

chosen based on Akaike's information criterion with correction. Effects of parity, day, and parity and day interaction were analyzed. When necessary, means were compared by Fisher's least significant difference. The degrees of freedom were estimated using the Kenward-Roger method. The analyzes were performed using the PROC GLIMMIX of the Statistical Analysis System (SAS). All the statistical evaluations were performed considering 0.05 as the critical level of probability for the occurrence of the type I error.

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Table 1. Supplement provided to cows at 60-d pre-partum and forage chemical composition

Item	¹ Supplement	<i>Uruchloa decumbens</i>			
		Dry	Dry-rainy	Rainy	Rainy-dry
DM ²	-	384.8	270.5	266.9	258.1
OM ³	952.8	875.8	940.4	711.7	919.2
CP ³	36.2	63.5	81.5	90.4	78.4
NDF ³	143.3	704.8	674.8	658	681.4
iNDF ³	-	291.1	207.3	205.4	248.2
NDIN ⁴	-	25.2	21.5	27.8	26.5

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (apNDF), indigestible neutral detergent fiber (iNDF), insoluble neutral detergent nitrogen (NDIN).¹Supplement composition (as fed-basis): corn meal (41.2%), soybean meal (56.3%), urea:ammonium sulfate (2.5%).

²g/kg of natural matter

³g/kg DM

⁴g/kg total nitrogen

Table 2. Performance of calves and their dams according to parity order in Nellore cows under grazing

Items	Parity			SEM	<i>P</i> -value	
	Nulliparous	Primiparous	Multiparous		Par	
iBW	443.0b	437.5b	515.9a	19.20		0.002
fBW	461.8b	468.3b	530.1a	17.80		0.002
ADGpre	0.41	0.46	0.34	0.059		0.373
ADGpost	-0.30	-0.18	-0.29	0.179		0.788
ADGf	0.15	0.21	0.17	0.044		0.192
Calf iBW	30.5	33.1	32.8	1.579		0.326
Calf fBW	202.9	188.9	210.0	11.78		0.306
ADGpr	0.57	0.54	0.58	0.018		0.126
ADGpo	0.76	0.75	0.77	0.039		0.821
ADGfc	0.72	0.66	0.74	0.032		0.146

iBW – inicial body weight of the cows (kg); fBW – final body of the cows (kg); ADGpre – average daily gain pre-partum (kg/d); ADGpost – average daily gain post-partum (kg/d); ADGf – final average daily gain (kg/d); ADGpr – average daily gain from birth to start of creep-feeding (kg/d); ADGpo – average daily gain from starts of creep-feeding until weaning (kg/d); ADGfc – final average daily gain (kg/d). Different letters declare significantly different between parities ($P < 0.05$).

Table 3. Milk production and composition according to parity order in Nellore cows under grazing

Items	Parity			SEM	Par	<i>P</i> -value	
	Nulliparous	Primiparous	Multiparous			Day	Par x Day
Milk yield, kg/d	6.5a	6.0a	7.2b	0.258	0.006	<0.001	0.882
Fat ¹	5.15	4.98	5.06	0.236	0.871	0.294	0.895
Protein ¹	3.26	3.18	3.34	0.078	0.317	<0.001	0.689
Lactose ¹	4.56	4.61	4.68	0.045	0.169	0.238	0.536
Total solids ¹	13.91	13.81	14.13	0.248	0.613	0.019	0.770

Different letters declare significantly different between parities ($P < 0.05$).¹g/100g

Table 4. Metabolites and hormones concentrations according to parity order in Nellore cows under grazing

Items	Parity			SEM	Par ²	P-value	
	Nulliparous	Primiparous	Multiparous			Day ³	Par x Day
Glucose, mg/dL	65.66	63.93	64.32	1,895	0.485	<0.001	<0.001
Triglycerides, mg/dL	37.47	40.35	38.60	1.450	0.332	<0.001	0.711
Total cholesterol, mg/dL	136.49	138.14	134.31	7.842	0.868	<0.001	0.367
VLDL, mg/dL	9.35	10.08	9.67	0.404	0.416	<0.001	0.622
LDL, mg/dL	44.62	46.49	40.61	6.550	0.628	<0.001	0.610
HDL, mg/dL	83.19	81.97	84.52	2.403	0.742	<0.001	<0.001
NEFA, mmol/L ¹	0.08	0.07	0.11	0.141	0.402	<.0001	0.936
βHB, mmol/L ¹	0.43	0.44	0.48	0.002	0.872	<0.002	0.023
Total proteins, g/dL	6.53c	6.91b	7.24a	0.178	0.002	<0.001	0.459
Albumin, g/dL	2.89b	2.87b	3.05a	0.067	0.034	<0.001	0.790
Globulins, g/dL	3.77b	4.07a	4.20a	0.294	0.021	<0.001	0.856
SUN, mg/dL	24.62a	24.78a	21.90b	1.473	0.023	<0.001	0.206
Creatinine, g/dL	1.24	1.22	1.27	0.046	0.355	<.0001	0.028
IGF-1, ng/mL	128.73	142.08	149.18	8.194	0.184	<0.001	0.01
Insulin, uIU/mL	2.31	2.78	2.89	0.416	0.511	<0.001	0.816
T3, ng/mL	2.49	2.53	2.43	0.368	0.971	0.112	0.819
T4, ug/dL	8.4	7.55	7.97	0.839	0.764	0.059	0.983
Progesterone, ng/mL	5.36	4.77	4.16	1.099	0.703	<0.001	0.537

Different letters declare significantly different between parities (P<0.05).

¹Non-esterified fatty acids (NEFA); β-hydroxybutyrate (βHB); Serum urea nitrogen (SUN)

²Parity (Par)

³Day relative to calving (Day)

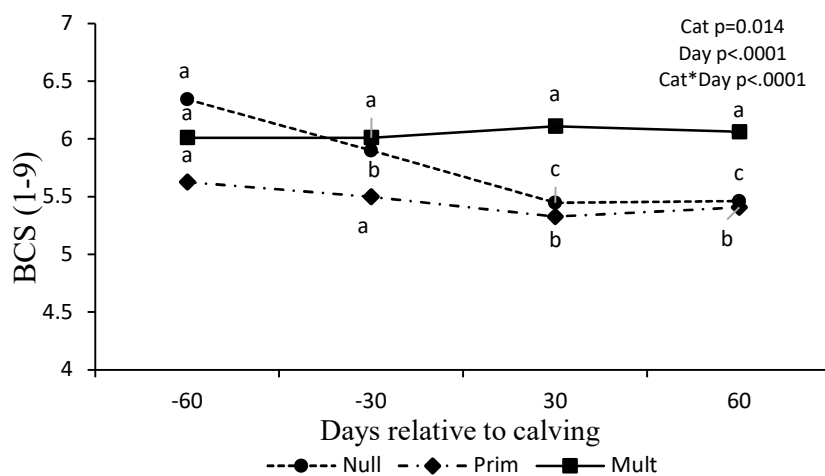
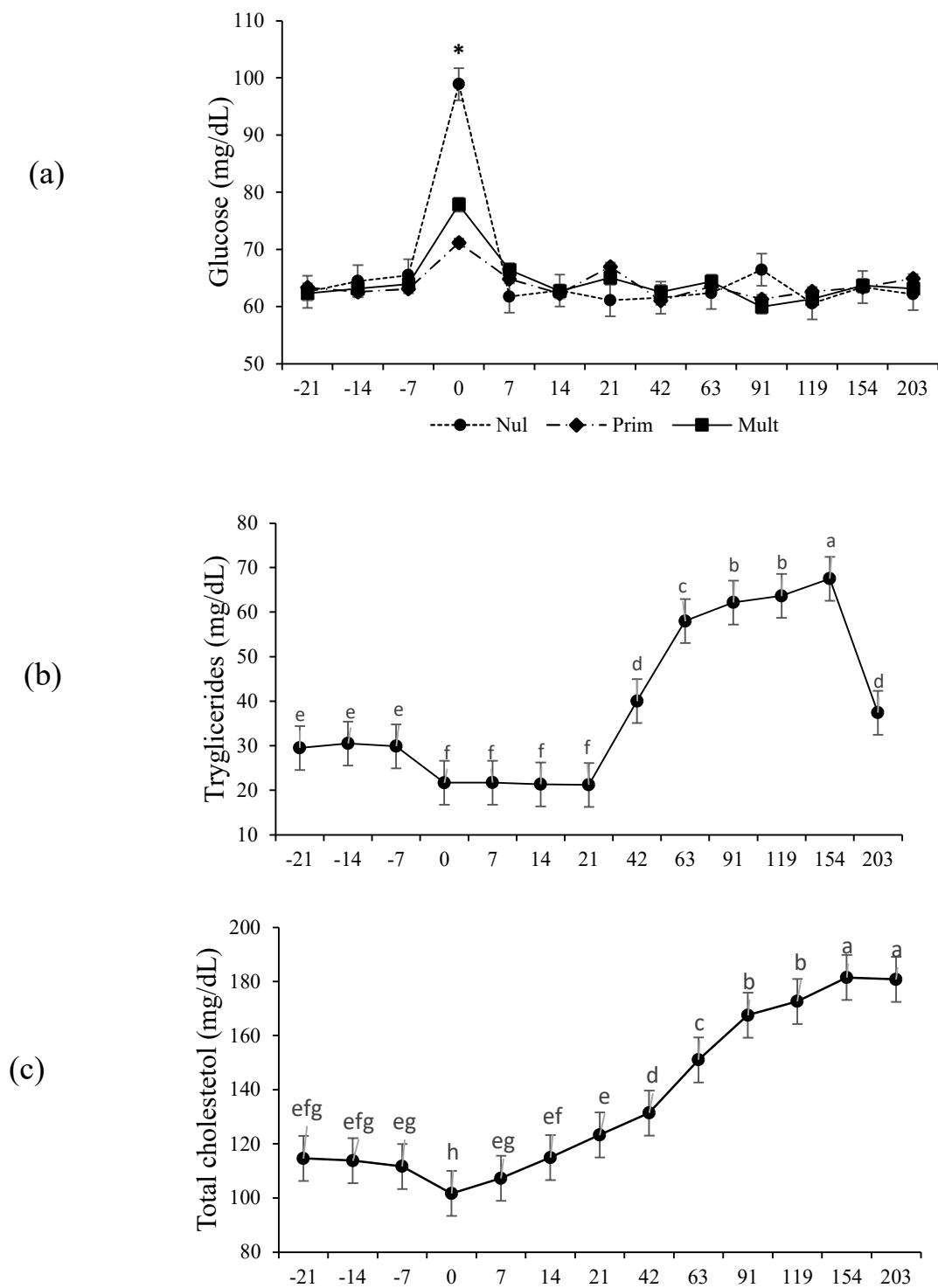


Figure 1. Body condition score (BCS) during of Nellore cows with different parity order under grazing according to the days relative to calving. Days followed by different superscripts within parity order are different ($P < 0.05$).



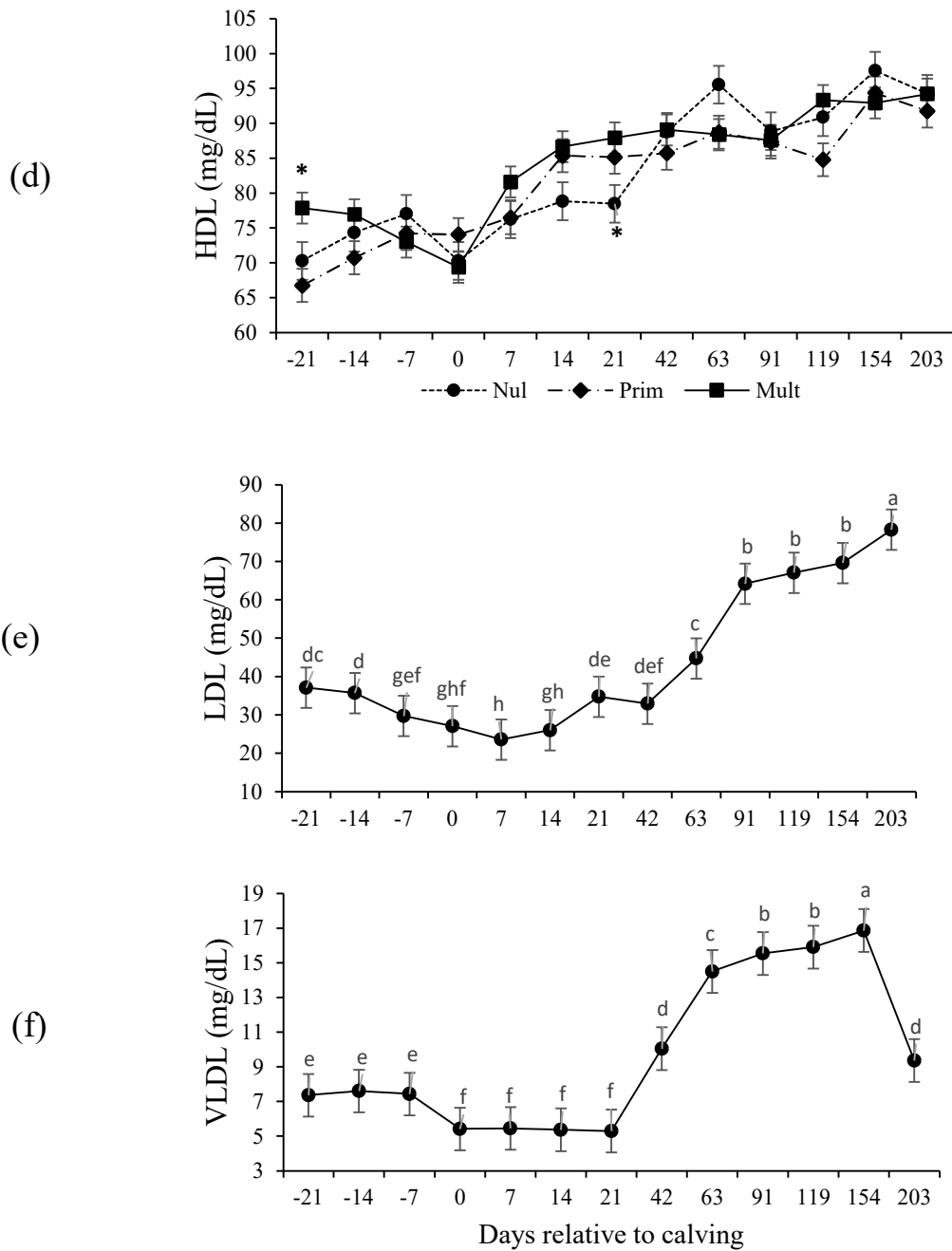


Figure 2. Glucose (a), triglycerides (b) total cholesterol (c), HDL (d), LDL (e), VLDL (f) serum concentrations in Nellore cows with different parity order under grazing according to the days relative to calving. Means with different superscripts differ from each other ($P < 0.05$). Days with *asterisks* (*) are significantly different between parities ($P < 0.05$).

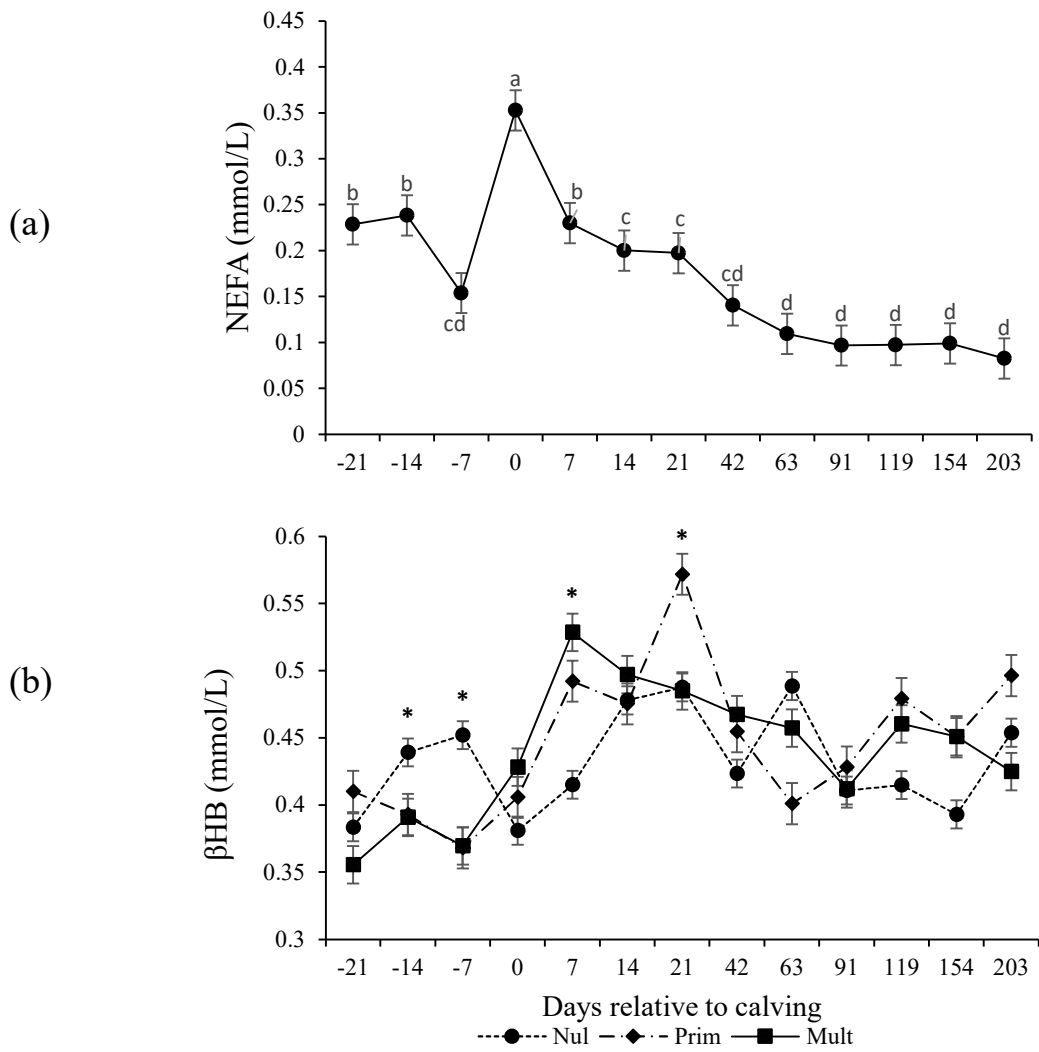


Figure 3. Non-esterified fatty acids (NEFA; a) and β -hydroxybutyrate (β HB; b) serum concentrations in Nellore cows with different parity order under grazing according to the days relative to calving. Means with different superscripts differ from each other ($P < 0.05$). Days with *asterisks* (*) are significantly different between parities ($P < 0.05$).

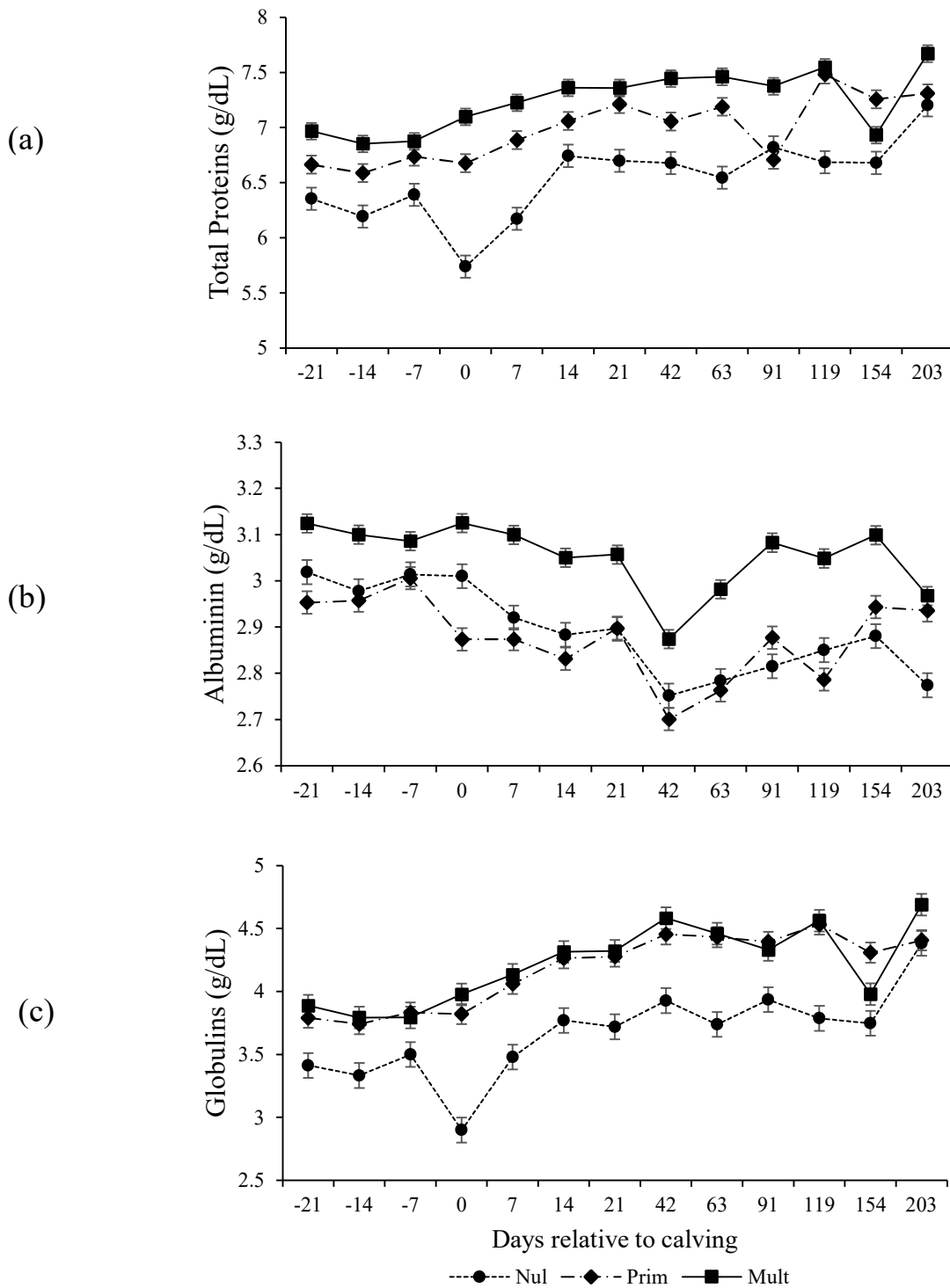


Figure 4. Total protein (a), albumin (b), and globulin (c) serum concentrations in Nellore cows with different parity order under grazing according to the days relative to calving.

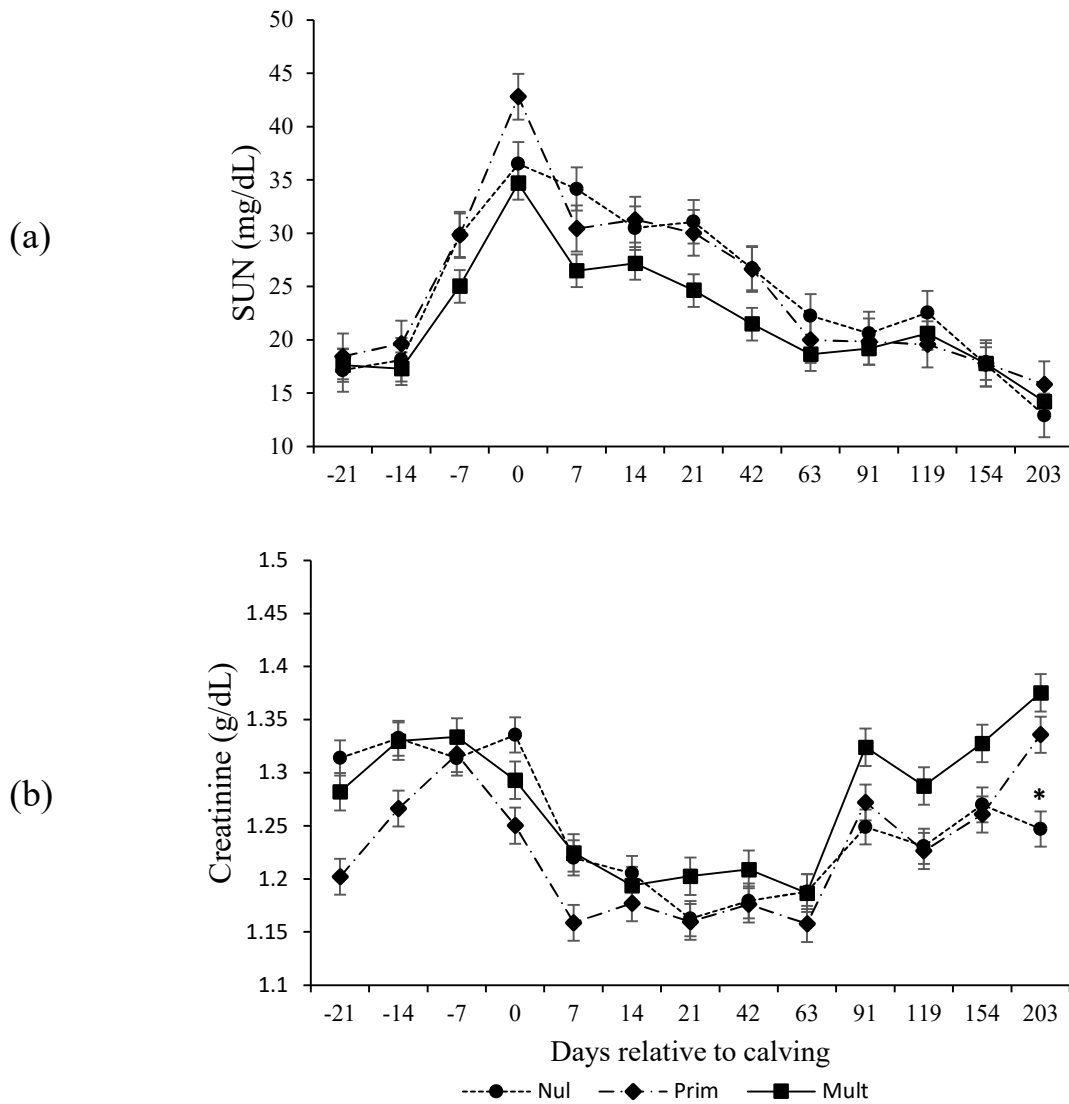


Figure 5. SUN (a) and creatinine (b) serum concentrations in Nellore cows with different parity order under grazing according to the days relative to calving. Days with *asterisks* (*) are significantly different between parities ($P < 0.05$).

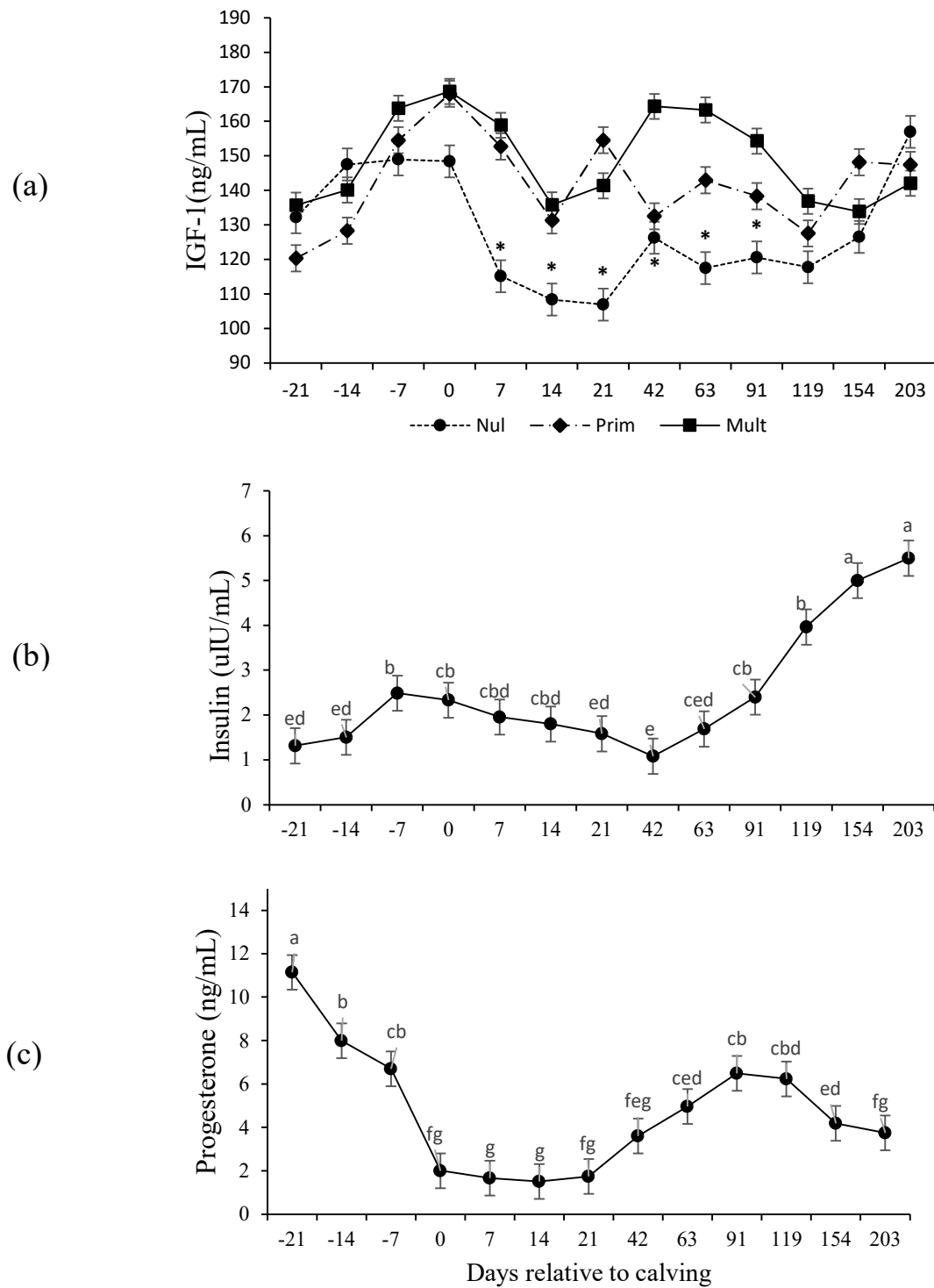


Figure 6. IGF-1 (a), insulin (b) and progesterone (c) in Nellore cows with different parity order under grazing according to the days relative to calving. Means with different superscripts differ from each other ($P < 0.05$). Days with *asterisks* (*) are significantly different between parities ($P < 0.05$).

CHAPTER 2 - Evaluation of nonlinear models to predict potential milk yield of beef cows according to parity order under grazing - Article published at the *Frontiers in Veterinary Science* (2021) - doi.org/10.3389/fvets.2021.721792

Abstract

This study aimed to evaluate the effect of parity order on milk yield (MY) and composition over time of grazing beef cows and to evaluate nonlinear models to describe the lactation curve. Thirty-six pregnant Nellore cows (12 nulliparous, 2 years; 12 primiparous, 3 years; and 12 multiparous, 4–6 years) were included in the study. Assigning calving day as day 0, milking was performed using a milking machine to estimate MY on days 7, 14, 21, 42, 63, 91, 119, 154, and 203. Dummy variable analyses were applied to estimate its effects on MY, composition (kg and percentage), afternoon/morning, and afternoon/total proportions. Since multiparous had higher MY than nulliparous and primiparous, two different groups were used for lactation curve analysis: Mult (multiparous) and Null/Prim (nulliparous and primiparous). The MY estimated by the BR-Corte (2016) (Nutrient Requirements of Zebu and Crossbred Cattle) equation was compared with the observed values from this study. Five nonlinear models proposed by Wood (1967) (WD), Jenkins & Ferrell (1984) (JF), Wilmink (1987) (WK), Henriques et al., (2011) (HR) and Cobby & Le Du (1978) (CL) were evaluated. Models were validated using an independent dataset of multiparous and primiparous cows. The estimates for the parameters a, b, and c of the CL equation were compared between groups and the BR-Corte equation using model identity methodology. Nulliparous and primiparous displayed similar MY ($P>0.05$), however, multiparous cows had an average MY of 0.70 kg/d greater than nulliparous and primiparous ($P<0.05$). Milk protein and total solids were higher for multiparous cows ($P<0.05$). Effect of days in milking was found for milk fat, protein, and total solids ($P<0.05$). The yield of all milk components was higher for multiparous than nulliparous and primiparous. The afternoon/morning and afternoon/total proportion of milk production were not affected by parities and days in milking ($P>0.05$), with an average of 0.76 and 0.42, respectively. The BR-Corte equation did not estimate correctly MY ($P<0.05$). The equations of WD, WK, and CL had the best estimative of MY for both Mult and Null/Prim datasets. The equations had very similar Akaike's information criterion with correction and mean square error of prediction.

Keywords: beef cattle; *Bos indicus*; lactation; milk composition; milk production;

Introduction

The milking ability of beef cows is one of the main factors influencing the weaning weights of calves (1), thus, many methods have been used to attempt to estimate beef cows' milk production and its influence on calf preweaning growth (2–4). The evaluation of milk production is also necessary to estimate the nutrient requirements of cows and calves since the nutritional balance of a lactating cow is important in the calculation of energy and protein requirements (5, 6). Thus, if it is over or underestimated, systematic errors can impair the estimation of nutrient requirements.

Milk yield can be estimated by different methods that include: determining differences in calf weights before and after suckling (2, 7, 8), hand milking (3, 4), or machine milking procedures (9–11). Those methods are possible with small numbers of animals but are not adaptable to larger herds. Such procedures, particularly those with a controlled suckling period before the calf separation as described by Boggs et al. (1), require repeated, intensive animal handling in which timing is critical. Furthermore, in general, data collection and handling procedures of grazing animals, specifically pregnant Nellore cows, is laborious since animals have a poor temperament and require extreme care. This challenge would explain the low number of studies and the low number of data points to fit an equation of milk production for grazing beef cows.

In the last BR-Corte edition (Third edition of the Brazilian tables of *Nutrient Requirements of Zebu and Crossbred Cattle*; 12), the estimated milk yield for Nellore cows during seven months of lactation was based on the model proposed by Cobby & Le Du (13). The equation was generated using data from feedlot cows, but the authors employed independent data from experiments with grazing Nellore cows to validate the equation. Although the equation provided a better estimate of milk yield than previous editions (14), none of these experiments explicitly evaluated the milk yield of different parity orders under grazing conditions, and there is still limited data in this regard.

Previous studies have shown that age and parity order can influence the metabolism and milk production of dairy (15, 16) and beef cows (6, 17), where primiparous display lower milk production and more unbalanced nutritional status compared to multiparous cows (15, 17, 18). Thus, different equations are needed to predict the milk yield of Nellore cows according to parity order, since they may differ in milk potential.

Therefore, this study aimed to evaluate the effect of parity order on milk yield and composition over time of grazing beef cows and to evaluate nonlinear models to describe the lactation curve.

Materials and Methods

The Animal Care and Use Committee at the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 120/2018) approved all animal care and handling procedures. Animals used in this study were provided by the UFV/Beef Cattle Research and Extension Unit, Viçosa-MG, Brazil, where the study was conducted from July 2018 to May 2019.

Experimental design and animals

Thirty-six pregnant Nelore cows (12 nulliparous, 12 primiparous, and 12 multiparous) were included in the study, with the following average age, body weight (BW), and body condition score (BCS; 1–9): 2 years, 442 (\pm 62) kg, 6.20 (\pm 0.5); 3 years, 457 (\pm 58) kg, 5.68 (\pm 0.5); 4–6 years, 505 (\pm 60) kg, 5.92 (\pm 0.5), respectively. This study lasted from 60 days prepartum to 203 days of the lactation period (two weeks before weaning).

The nomenclature for each category related to parity was set at the beginning of the experiment (late gestation period) and used throughout the manuscript. Even though after calving the parity order changed (e.g., nulliparous became primiparous cows), the nomenclature remained constant along the manuscript.

Parity classes were systematically randomized into six paddocks, assuming two cows from each parity class in the paddocks (thus characterizing sub-blocks). The animals were assigned to paddocks 15 days before the beginning of the experiment to acclimate to the environment and the herd. The average area of the paddocks was 7 hectares, covered with *Urochloa decumbens* grass, and cows had free access to water and feeders.

All cows were group-fed with an energy-protein supplement (1.0 kg/d) with 35% crude protein (CP) for 60 days pre-partum (41.2% corn meal, 56.3% soybean meal, and 2.5% urea:ammonium sulfate). The supplement was calculated to supply approximately 40% of the cows' protein requirements, as recommended by the BR-Corte (12). We provided a linear trough space of 0.70 m per cow to ensure homogeneous supplement consumption among groups. The supplement was supplied at 12:00 pm to minimize any interference from animal grazing behavior.

After calving, cows remained in the same paddocks with their respective calves. A commercial mineral mix (CaHPO₄ = 50.00%; NaCl = 47.775%; ZnSO₄ = 1.4%; Cu₂SO₄ = 0.70 %; CoSO₄ = 0.05%; KIO₃ = 0.05% and MnSO₄ = 0.025%) was also offered to cow-calf pairs for *ad libitum* consumption throughout the experiment, supplied separately in additional feeders.

After 90 days of age, calves were offered 5 g/kg BW of an energy-protein supplement formulated to contain 20% CP in a creep-feeding system until the end of the study (203 days in milk).

Milk collection and analyses

Calving day was assigned as day 0, and milking was performed using a milking machine to estimate milk yield on days 7, 14, 21, 42, 63, 91, 119, 154, and 203.

Milking procedures were as described by Boggs et al. (1), providing a controlled suckling period before the calf separation. To empty udders, calves were separated from their dams at 3 pm, and then reunited at 5:45 pm and allowed to suckle until 6 pm, when they were once again separated. The first milking was performed at 6:00 am on the next day after an injection of 10 IU (international unit) of oxytocin (10 IU/mL; Ocitovet®, Brazil) in the cow's mammary vein, and the produced milk was weighed. The exact time when the milking of each cow ended was recorded. After morning milking, cows were kept separated from their calves until the end of the afternoon milking, which was performed at 6 pm. Then, the total daily production was calculated by the sum of both milkings to obtain a 24-hour milk production.

From each cow, a 30 mL sample of milk was collected at morning and afternoon milking to evaluate milk composition. Samples were stored at 4°C in a refrigerator, each receiving one bronopol tablet per sample as a preservative for further analyses. Milk samples were analyzed fresh for percentage of protein, fat, lactose, and total solids content using infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil). A weighted average was calculated for each component based on morning and afternoon milk yields.

Forage sampling and analyses

Every 30 days, grass samples were collected by hand-plucking to evaluate the forage selected by animals and collected by cutting at the ground level from five delimited areas (0.5 x 0.5 m), selected randomly in each paddock to quantify dry matter (DM) per ha. In these circumstances, all the samples were weighed, oven-dried (55°C), and then ground to pass through 1- and 2-mm screens in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA). All data from each month were combined and expressed as an average per season as follows: dry season = August (beginning of the experiment), dry-rainy transition = September to November; rainy season = December to February; rainy-dry transition = March to June (end of the experiment).

The forage and supplement samples were analyzed following the procedures described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; 19) for DM (method G-003/1), ash (method M-001/1), CP (method N-001/1), neutral detergent fiber corrected for ash and protein (aNDF; method F-002/1). Indigestible neutral detergent fiber (iNDF; 20) was processed at 2 mm and quantified by *in situ* incubation procedures with nonwoven textile bags (100 g/m²) for 288 h.

Statistical Analyses

Variables measured during lactation were analyzed using the following model:

$$Y_{ijkl} = \mu + C_j + P_{k(l)} + e_{ijkl} \quad [1]$$

where: Y_{ijkl} = observation measured on animal i , belonging to parity class j , within paddock k and sub-block l ; μ = overall mean; C_j = parity effect j (fixed); $P_{k(l)}$ = paddock effect k with the nested sub-block l (random); and e_{ijkl} = residual random effect, assumed to be independent and identically distributed (0, σ^2_e). The covariate days in milk was added to this model when its effect was significant.

Given the ordinal nature of parity class (0, 1, and 2), dummy variable analyses (21) were applied to estimate its effects on milk yield, composition, afternoon/morning, and afternoon/total proportions. For this, the mixed model presented in [1] was fitted by using PROC MIXED of SAS[®] software assuming the dummy variable as covariates. The parity effect estimates were added to the overall mean to report the results on the same scale as the observed data.

Based on the model [1], since multiparous had higher milk yield than nulliparous and primiparous, two different groups were used for lactation curve analysis: Mult (multiparous cows) and Null/Prim (both nulliparous and primiparous categories together).

The milk yield estimated by the BR-Corte (12) equation was compared with the observed values from this study using the Model Evaluation System (MES version 3.2.2) using the following regression model:

$$y = \beta_0 + \beta_1 \times X$$

where x = predicted values; y = observed values; β_0 and β_1 = intercept and slope, respectively.

The regression was evaluated according to the following statistical hypothesis:

$$H_0: \beta_0 = 0 \ \& \ \beta_1 = 1 \ \text{and} \ H_a: \text{not } H_0$$

Estimates were evaluated using the estimated value of the mean square error of the prediction and its components (22):

$$MSEP = SB + MaF + MoF = 1/n \sum_{i=1} (x_i - y_i)^2 ,$$

$$SB = (x - y)^2 ,$$

$$MaF = (S_x + S_y)^2 ,$$

$$MoF = 2S_x S_y (1 - r) ,$$

where x are the predicted values; y are the observed values; $MSEP$ is the mean squared error of prediction; SB is the squared bias; MaF is the magnitude of random fluctuation; MoF is the model random fluctuation; S_x and S_y are the standard deviations of the predicted and observed values, respectively, and r is the Pearson linear correlation between the predicted and observed values.

For all calculations of variance and covariance, the total number of observations was used as a divisor because it was an estimate of the prediction error (22). The prediction of efficiency was determined by estimating the correlation and concordance coefficient (CCC) or reproducibility index described by Tedeschi (23).

The CCC indicates models with good accuracy and precision (when close to 1.0) or models with a problem of reproducibility (when close to 0.0). The smallest mean square error of prediction indicates the best model in the evaluation. In this study, it can indicate that the model error is associated with the squared bias (SB) or errors related to the high dispersion of data around the mean (MaF) or systematic errors concerning the direction of the curve predicted (MoF).

Five nonlinear different equations forms were fitted to the dataset Mult and Null/Prim:

$$\begin{aligned}
[\text{CL}] \text{ Cobby \& Le Du (13)} \quad Y_t &= a + bt - a \exp(-ct) + e_t \\
[\text{WD}] \text{ Wood (24)} \quad Y_t &= a^t b \exp(-ct) + e_t \\
[\text{WK}] \text{ Wilmink (25)} \quad Y_t &= a - b \exp(-ct) - dt + e_t \\
[\text{JF}] \text{ Jenkins \& Ferrell (26)} \quad Y_t &= a t \exp(-ct) + e_t \\
[\text{HR}] \text{ Henriques et al., (27)} \quad Y_t &= a + b t \exp(-ct) + e_t
\end{aligned}$$

where: Y_t = milk yield (kg/d) from lactating beef cows; t = time of lactation (weeks), \exp = exponential of natural logarithm; a = theoretical initial yield (production scale) (WD, CL, WK, JF, HR), b = decrease rate of production after peak of lactation (CL), increase rate of production up to peak of lactation (WD, HR, WK), c = increase rate of production up to peak of lactation (CL), decrease rate of production after peak of lactation (WD, JF, HR), d = decrease rate of production after peak of lactation (WK); e_t = residual term, assumed as NIID ($0, \sigma^2_e$).

Wood (24) is the most widely applied equation for dairy cattle lactation curve. Jenkins & Ferrell (26) proposed a similar equation to WD but removing the parameter b . Henriques et al., (27) equation is based on JF model with the addition of a parameter for adjustment for the beginning of lactation. Wilmink (25) is a modification of the lactation curve function in CL.

The parameters of the functions were estimated through the procedure NLMIXED of SAS (SAS Inst. Inc., Cary, NC - version 9.3) and were adjusted by the Gauss-Newton method.

An independent database (Rodrigues, 2021 - unpublished data) of Nellore cows from the UFV/Beef Cattle Research and Extension Unit was used for training the models which were composed of 130 and 112 observations of multiparous ($n=16$) and primiparous ($n=14$) cows, respectively, with eight lactation points each (1, 2, 4, 6, 9, 13, 20, 29 weeks). The milking procedures were performed using the exact same approach as the present study: milking twice (morning and afternoon) with a controlled suckling period before the calf separation. The predicted values of the alternative equations generated were compared to the external data values milk yield using the Model Evaluation System (MES version 3.2.2). Akaike's information criterion with correction (AICC), MSE, and the distribution of the error (SB, MaF, MoF) were used to choose the best models.

Milk production upon lactation peak and time until peak was calculated for a 30-week lactation length by the derivative of the equations equal to zero.

The estimates for the parameters a , b , and c from the Cobby & Le Du (13) model was compared between groups and the BR-Corte equation (12) (current equation to estimate milk

yield of Nellore cows) using model identity methodology based on overlapping of asymptotic confidence intervals (21).

All the statistical evaluations were performed considering 0.05 as the critical level of probability for the occurrence of the type I error.

Results

The average dry matter (DM) yield was expressed per period (season) during the experiment as follows: dry season = 4.69 t/ha, dry-rainy transition = 4.33 t/ha; rainy season = 2.93 t/ha; rainy-dry transition = 3.74 t/ha (Figure 1). Forage chemical composition by season is presented in Table 1.

Milk yield was different between parities ($P < 0.05$). Nulliparous and primiparous displayed similar milk yield ($P > 0.05$). However, multiparous cows had an average milk yield 0.70 kg/d greater than nulliparous and primiparous. The effect of days in milking was significant ($P < 0.05$) and was estimated as -0.008 kg per day, which is a decrease in production per day (Table 2).

Milk fat did not differ between parities ($P > 0.05$); however, there was an effect of days in milking, in which milk fat increased linearly 0.001% per day ($P < 0.05$; Figure 1a). Milk lactose was on average 4.52% and did not differ between parities or days in milking ($P > 0.05$; Table 2).

Milk protein and total solids were different between parities ($P < 0.05$; Table 2), higher for multiparous cows (0.11 and 0.31%, respectively). Days in milk also affected these variables, as they increased linearly 0.001 and 0.003% per day, respectively (Figure 2b and 2d).

However, the yield of milk components expressed in kg (fat, protein, lactose, and total solids yield) were all higher for multiparous cows ($P < 0.05$), while nulliparous and primiparous displayed similar yields ($P > 0.05$, Table 2).

For multiparous cows the intercept for fat, protein, lactose and total solids yield were 0.367, 0.242, 0.328 and 0.932, respectively, and for nulliparous and primiparous cows 0.317, 0.283, 0.242, 0.866, decreasing linearly 0.0003, 0.0002, 0.0004, 0.0009 per day, respectively ($P < 0.05$, Figure 3). The afternoon/morning and afternoon/total proportion of milk production were not affected by parity or days of milking ($P < 0.05$), with an average of 0.76 and 0.42, respectively (Table 2).

Based on the milk yield differences between parities, two different groups were used for lactation curve analysis: Mult (multiparous cows) and Null/Prim (both nulliparous and primiparous categories together).

The BR-Corte equation did not estimate milk yield of the dataset correctly because the intercept and slope did not differ from zero and 1, respectively ($P < 0.05$). The BR-Corte equation demonstrated overprediction, with 53 and 67% of the MSEF related to model bias (Table 3), which shows the need for the development of new equations to estimate milk yield of grazing Nellore cows.

Therefore, five nonlinear alternative equations forms were fitted to the dataset Mult and Null/Prim: CL, WD, WK, JF, and HR (Figures 4 and 5).

The equation JF fitted for Mult and Null/Prim did not estimate milk yield of the independent data correctly because the intercept and slope did not differ from zero and 1, respectively. In addition, both equations had the highest AICC and MSEF, showing that the models did not adjust to the independent data (Tables 4 and 5).

The HR equation did not estimate milk yield of the independent data for Null/Prim equation because the slope did not differ from 1 ($P < 0.05$; Table 5). Even though the HR equation fitted well to the Mult data, we did not consider it as a plausible equation form for use, as it did not adjust also to Null/Prim dataset.

The equations of WD, WK, and CL had the best estimative of milk yield and description of the lactation curve of grazing Nellore cows for both Mult and Null/Prim datasets (Tables 4 and 5). The equations had very similar AICC and MSEF, with 96% for Mult and around 92 to 96% for Null/Prim of the MSEF associated with random error (MoF). All three equations were resembling in estimate mean milk yield, hence, the choice of any of those would not impact negatively the estimative of milk yield.

The estimated milk yield at the peak of production and time until peak for multiparous and Null/Prim according to equations form are:

WD = 7.49 kg at 5.90 weeks for Mult, and 6.45 kg at 5.72 weeks for Null/Prim.

WK = 7.56 kg at 5.82 weeks for Mult, and 6.08 kg at 6.47 weeks for Null/Prim.

CL = 7.55 kg at 3.30 weeks for Mult, and 6.45 kg at 2.62 weeks for Null/Prim

The parameters a, b and c, of the CL equation generated for Mult and Null/Prim differ from the BR-Corte parameters (Figure 6).

Discussion

Nulliparous and primiparous are in a different physiological state than multiparous cows since they are not physically mature at this age (28). Therefore, a difference in milk production was expected, however, it was not known the magnitude of the differences.

Our outcome reveals an average milk yield that was approximately 11% lower (-0.70 kg/d) for nulliparous and primiparous cows than multiparous Nellore cows. These results are consistent with other studies evaluating *Bos taurus* dairy (15, 16) and beef cows (6, 29). However, the range of the differences in milk yield according to age or parity, as well as the lactation peak and persistence, may vary between species (such as *Bos taurus* and *Bos indicus*) and breed crosses.

In the *Nutrient Requirements of Beef Cattle* - NASEM (2016), the equation used to predict milk yield of beef cows includes an age coefficient that represents 26% and 12% less milk yield for cows of ≤ 2 years and > 2 but ≤ 3 years, respectively (6). Also, according to NASEM (2016), the peak lactation of beef cows (based on a wide variety of *B. taurus* breed and breed crosses) occurs approximately at 8.5 weeks (6). In lactating dairy cows, milk production usually peaks at 4 to 8 weeks postpartum (16). In contrast to both studies, for Nellore cows, we found that the peak of lactation is substantially earlier (around 3 to 6 weeks) than that observed for *B. taurus* dairy and beef cows. There is no mention of differences in the milk yield and time to peak of lactation according to the parity or age of Nellore cows in the BR-Corte editions (12, 14).

Greater milk production of multiparous cows may be explained by the largest balance of net energy for milk production, since this category nutritional requirements are only for maintenance and milk production, unlike young that cows still have nutritional growth requirements. Also, as nulliparous still require nutrients for their continued growth, the mammary gland is not completely developed, and the capacity of milk production is reduced compared to multiparous cows. In dairy cows, although the underlying mechanisms are not well understood, several indicators suggest that the mammary gland is more metabolically active in multiparous cows (i.e., higher expression of genes related to metabolic activity) than in primiparous cows, especially at the onset and peak of lactation (30). This observation suggests, at least in part, that the lower milk production observed in primiparous cows could be related to a lower density of secretory cells. Generally, multiparous animals have a higher milk yield but lower lactation persistency than younger animals (30–32). In this experiment,

besides the lower milk yield, the lactation curve of nulliparous and primiparous cows was indeed much flatter than that of multiparous cows.

The milk composition of Nellore cows in BR-Corte (12) was 15.0% total solids, 4.58% lactose, and 5.61% fat as the average milk composition from Nellore cows during all lactation periods. However, milk protein increased during the lactation period from 3.57% (4th week) to 3.97% (28th week). In the present study, all milk components changed during the lactation period except for lactose. Total solids, protein, and fat increased linearly throughout lactation. However, when milk composition is expressed in kg per day, all components yield decreased throughout lactation because the percentage of milk yield reduction was greater than the solids increase. The greatest fat percentage value observed in BR-Corte (12) may be related to a higher energy diet since the cows were maintained in a feedlot system and fed corn silage, which also impacted the milk total solid values. Milk composition can vary according to breed and diet; however, in general, data from previous studies show a percentage of milk fat ranging from 4.5 to 5% for Nellore cows (10, 11, 33).

Reduced milk protein and total solids for nulliparous cows were expected. They may be explained by the differences in protein metabolic status since nulliparous has lower blood albumin and total protein than multiparous beef (17) and dairy cows (34, 35). These differences are even higher when milk components were expressed in kg since parities also differ in milk yield.

Over the years, several models and approaches have been used to describe the lactation curve of cattle (13, 24-27, 36, 37). The equation most widely used for describing the lactation curve of dairy cows is the model proposed by Wood (24); however, its use for beef cattle milk production has been limited since it demands a relatively large number of data points to fit the equation. Therefore, NASEM (2016) used a similar equation proposed by Jenkins and Ferrell (26) that requires fewer data points (6).

In the study of Henriques et al. (27), the best equations to estimate milk yield of Nellore cows were based on Wood (24) and a modification of Jenkins and Ferrell (26) equation. However, the method of milk collection was weigh-suckle-weigh, then multiplying the morning yield by 2 to estimate daily milk yield, which has been criticized due to data variation and wrong daily estimative. The model that fits better for describing a lactation curve for Nellore cows, according to the BR-Corte (12), is the one proposed by Cobby and Le Du (13) in which after peak milk yield tend to decline linearly. Although the equation

presented in BR-Corte (12) provides a better estimation than the past editions, there are still limitations on its database.

Studies regarding milk production of Nellore cows are often conducted in pens and using diets that mischaracterize the range cattle system, as well as methods of milking that can over or underestimate the milk production depending on the approach. Therefore, the BR-Corte model overprediction was expected because the equation was based on a study developed in a feedlot system using corn silage as a roughage source (38). Although the experiment validated the equation with independent data of studies with grazing Nellore cows, in all of these studies (including the observed values), the cows were milked once in the morning, and then this value was doubled to estimate daily production or extrapolated the morning milk yield for a 24 h-period. We believe that this overestimation is also related to the equivocated estimation of milk yield based on only one milking because the morning and afternoon yields contribute to the total daily production in different ratios (10, 39). Based on this rationale, the development of new equations that correctly estimate milk production on a grazing range system was necessary. Those explanations are supported by the analysis of model identity that showed that the parameters of the CL equations created compared with the BR-Corte were indeed different, even for the multiparous equation, which used the same animal category as the BR-Corte database.

The equations of WD, WK, and CL were the best in estimating milk yield and describing the lactation curve of both Mult and Null/Prim datasets. All three equations were similar in estimate mean milk yield and milk production upon lactation peak. The only difference between the equations is the occurrence peak of production, in which CL equation estimates an earlier peak of production than the WD and WK equation forms. In terms of using milk yield information for energy and protein requirements, choosing any of those would not impact negatively the data estimative.

In this experiment, morning and afternoon yield indeed contributed at different ratios to the total daily production. However, the values are not consistent with other studies (10, 39). In addition, we did not find any effect of days in milking for these ratios. Thus, it can be assumed that only one ratio for the whole lactation is needed to estimate total daily milk production, based only on the morning milking.

Conclusions

Parity influences the milk production and composition of beef cows. Milk yield can be estimated based on WD, WK and CL equation forms, using different equations for multiparous (4–6 years) and nulliparous/primiparous (2–3 years), with an estimated average peak of milk production of 7.5 and 6.3 kg, respectively, and time until peak ranging from 3 to 6 weeks.

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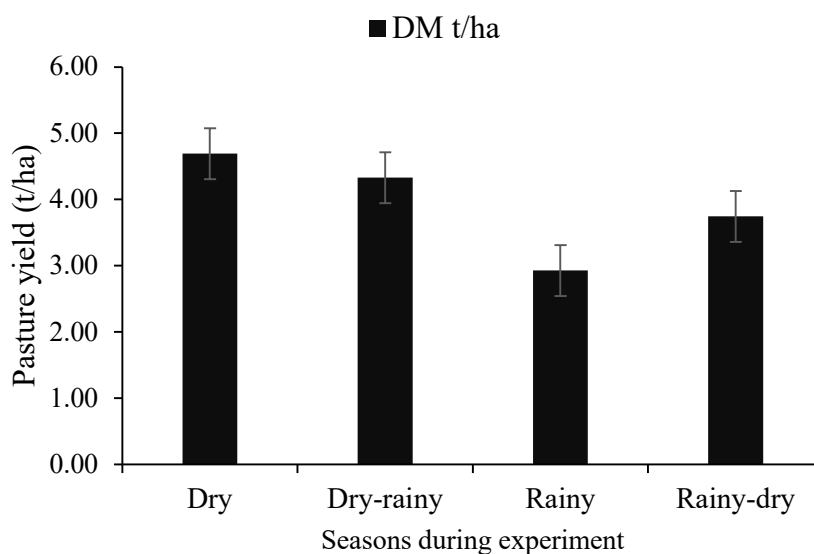


Figure 1. Pasture yield during experiment period per season. Dry season = August (beginning of the experiment), Dry-rainy transition = September to November; Rainy season = December to February; Rainy-dry transition = March to June (and of the experiment). DM: dry matter; t/ha: ton per hectare.

Table 1. Forage chemical composition

Items	<i>Uruchloa decumbens</i>			
	Dry	Dry-rainy	Rainy	Rainy-dry
DM ¹	384.8	270.5	266.9	258.1
OM ²	875.8	940.4	711.7	919.2
CP ²	63.5	81.5	90.4	78.4
apNDF ²	704.8	674.8	658.0	681.4
iNDF ²	291.1	207.3	205.4	248.2
NDIN ³	25.2	21.5	27.8	26.5

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (apNDF), indigestible neutral detergent fiber (iNDF), insoluble neutral detergent nitrogen (NDIN).

^{1/} g/kg of natural matter

^{2/} g/kg DM

^{3/} g/kg total nitrogen

Table 2. Regression coefficients for the covariate days in milk (with respective P-values), estimates of parity order effects and general standard error (SE).

Items	Day	Parity order			SE
		Nulliparous	Primiparous	Multiparous	
Milk yield, kg/d	-0.0080 (<.0001)	6.5b ¹	6.3b	7.2a	0.223
Fat, %	0.0030 (<.0001)	4.80	4.90	5.02	0.130
Protein, %	0.0010 (<.0001)	3.21b	3.20b	3.37a	0.055
Lactose, %	0.0001 (0.496)	4.56	4.53	4.56	0.031
Total solids, %	0.004 (<.0001)	13.73b	13.86b	14.03a	0.163
Fat, kg	-0.0003 (<.0001)	0.316b	0.318b	0.367a	0.010
Protein, kg	-0.0002 (<.0001)	0.203b	0.203b	0.242a	0.008
Lactose, kg	-0.0004 (<.0001)	0.286b	0.280b	0.328a	0.011
Total solids, kg	-0.0009 (<.0001)	0.868b	0.864b	0.932a	0.026
Afternoon/Morning ²	0.0001 (0.786)	0.79	0.71	0.78	0.050
Afternoon/Total ²	0.0001 (0.261)	0.43	0.41	0.42	0.013

¹Parity order followed by the same letters in the row are statically equal by dummy variable-based F test at 5% of probability (significance level).

²Production ratios afternoon/morning and afternoon/total.

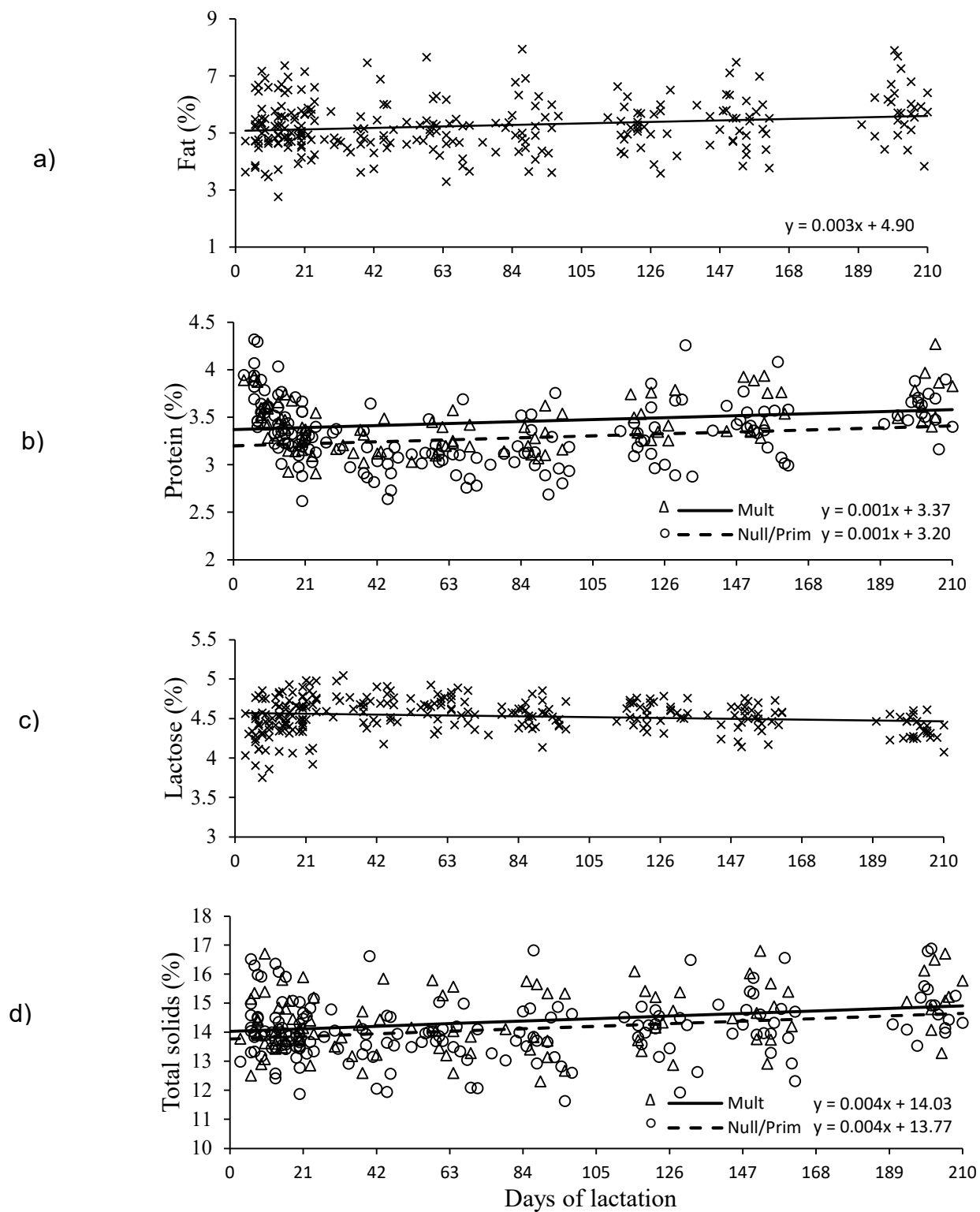


Figure 2. Milk fat (a), protein (b), lactose (c) and total solids (d) of Nellore cows according to parity order under grazing. Mult (multiparous), Prim (primiparous), Null (nulliparous).

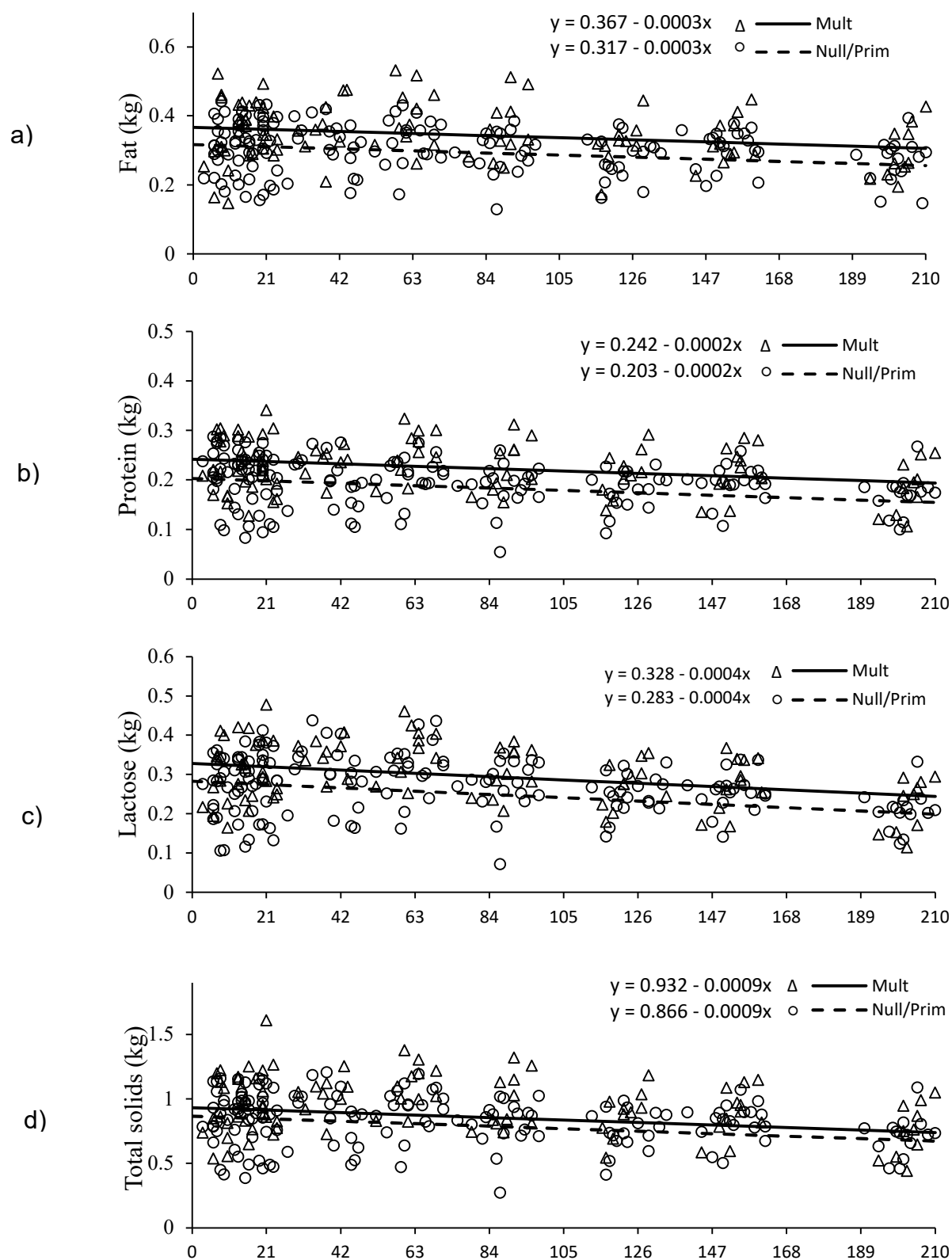


Figure 3. Fat yield (a), protein yield (b), lactose yield (c) and total solids yield (d) of Nellore cows according to parity order under grazing. Mult (multiparous), Prim (primiparous), Null (nulliparous).

Table 3. Mean (kg) and descriptive statistic of relationship among the observed and predicted values of milk production of Nellore cows according to parity order

Item	Multiparous		Nulliparous/Primiparous	
	OBS ^a	BR	OBS ^a	BR
Mean	6.60	7.98	5.94	7.98
Standard deviation	1.64	0.60	1.50	0.60
Maximum	10.26	8.67	9.19	8.67
Minimum	2.69	6.82	2.59	6.82
R^b	-	0.41	-	0.30
CCC ^c	-	0.16	-	0.08
Regression				
Intercept \pm SD ^d	-	-2.34 \pm 2.04	-	-0.25 \pm 1.51
Slope \pm SD	-	1.12 \pm 0.25	-	0.77 \pm 0.18
P-value ^e	-	<.0001	-	<.0001
MSEP ^d		4.10		6.29
SB	-	1.90	-	4.24
MaF	-	0.00	-	0.01
MoF	-	2.19	-	2.03

^aOBS—observed values; BR—predicted values in BR-Corte (2016).

^bR—correlation coefficient

^cCCC—correlation and concordance coefficient.

^dSD—standard deviation.

^e H_0 : $b_0=0$ & $b_1=1$.

^fMSEP—mean square error of prediction; SB—square bias; MaF—magnitude of random fluctuation; MoF—model random fluctuation.

Table 4. Equation parameters and descriptive statistic of relationship between the observed and predicted values of milk production of Multiparous Nellore under grazing

	OBS ^a	WD	HR	JF	WK	CL
<i>Parameters</i>						
a	-	6.507	5.074	3.475	8.614	7.927
b	-	0.184	1.224	-	3.240	-0.096
c	-	0.031	0.168	0.120	0.401	1.503
d	-	-	-	-	0.126	-
AICC ^b	-	303.3	305.8	445.6	305.5	309.4
<i>Descriptive statistics</i>						
Mean	6.96	6.67	6.75	8.09	6.73	6.70
SD ^c	1.61	0.74	0.77	0.58	0.78	0.77
Maximum	10.3	7.43	7.76	8.67	7.57	7.55
Minimum	3.50	5.00	5.35	6.82	4.97	5.15
Regression						
Intercept ± SD	-	1.90 ± 1.222	2.05 ± 1.200	7.49 ± 0.112	2.08 ± 1.162	2.27 ± 1.177
P-value ^d	-	0.122	0.089	<.0001	0.075	0.065
Slope ± SD	-	0.75 ± 0.182	0.72 ± 0.176	0.09 ± 0.016	0.72 ± 0.171	0.70 ± 0.174
P-value ^e	-	0.186	0.122	<.0001	0.112	0.088
CCC ^f	-	0.256	0.264	0.123	0.272	0.257
MSEP ^g	-	2.36	2.35	10.15	2.35	2.39
SB	-	0.085	0.040	4.038	0.055	0.070
MaF	-	0.031	0.044	5.856	0.046	0.053
MoF	-	2.253	2.270	0.258	2.245	2.273

^aOBS-independent values; ^bAICC – Akaike’s information criterion with correction; ^cSD–standard deviation;

^d $H_0: \beta_0=0$; ^e $H_0: \beta_1=1$; ^fCCC–correlation and concordance coefficient;

^gMSEP–mean square error of prediction; SB–square bias; MaF–magnitude of random fluctuation; MoF–model random fluctuation.

WD: Wood (1967); HR: Henriques et al., (2011); JF: Jenkins & Ferrell (1984); WK: Wilmink (1987); CL: Cobby and Le Du (1978).

Table 5. Equation parameters and descriptive statistic of relationship between the observed and predicted values of milk production of Nulliparous and Primiparous Nellore cows under grazing

	OBS ^a	WD	HR	JF	WK	CL
<i>Parameters</i>						
a	-	5.928	5.006	2.630	7.227	6.613
b	-	0.114	0.755	-	1.781	-0.053
c	-	0.020	0.172	0.131	0.320	2.129
d	-	-	-	-	0.081	-
AICC ^b	-	442.6	446.1	789.2	444.1	452.8
<i>Descriptive statistics</i>						
Mean	5.92	6.05	6.83	1.12	6.14	6.31
SD ^c	1.34	0.42	0.72	0.47	0.42	0.50
Maximum	8.80	6.45	7.76	1.72	6.48	6.85
Minimum	2.90	4.87	5.35	0.17	4.86	5.13
Regression						
Intercept ± SD	-	-1.05 ± 1.759	2.24 ± 1.182	5.47 ± 0.378	-0.82 ± 1.75	-0.59 ± 1.509
P-value ^d	-	0.549	0.060	<.0001	0.637	0.694
Slope ± SD	-	1.15 ± 0.290	0.53 ± 0.172	0.08 ± 0.070	1.11 ± 0.280	1.03 ± 0.238
P-value ^e	-	0.596	0.008	<.0001	0.689	0.887
CCC	-	0.203	0.180	0.015	0.199	0.238
MSEP ^g	-	1.58	2.57	24.71	1.59	1.64
SB	-	0.016	0.822	23.066	0.016	0.124
MaF	-	0.004	0.111	0.004	0.002	0.0003
MoF	-	1.560	1.641	1.635	1.571	1.523

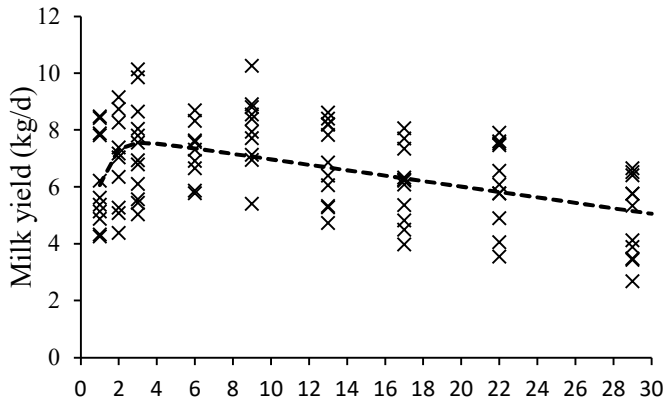
^aOBS-independent values; ^bAICC – Akaike’s information criterion with correction; ^cSD–standard deviation; ^dH₀: β₀=0; ^eH₀: β₁=1; ^fCCC–correlation and concordance coefficient;

^gMSEP–mean square error of prediction; SB–square bias; MaF–magnitude of random fluctuation; MoF–model random fluctuation.

WD: Wood (1967); HR: Henriques et al., (2011); JF: Jenkins & Ferrell (1984); WK: Wilmink (1987); CL: Cobby and Le Du (1978).

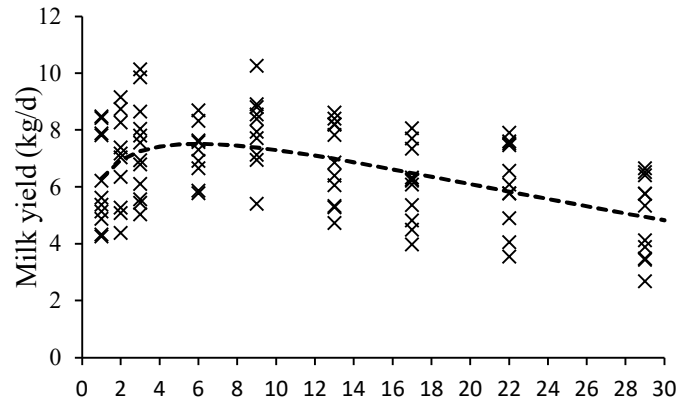
[CL] Cobby and Le Du (1978)

$$y = 7.927 - (0.0956 \times \text{week}) - 7.927 \times e^{(-1.502 \times \text{week})}$$



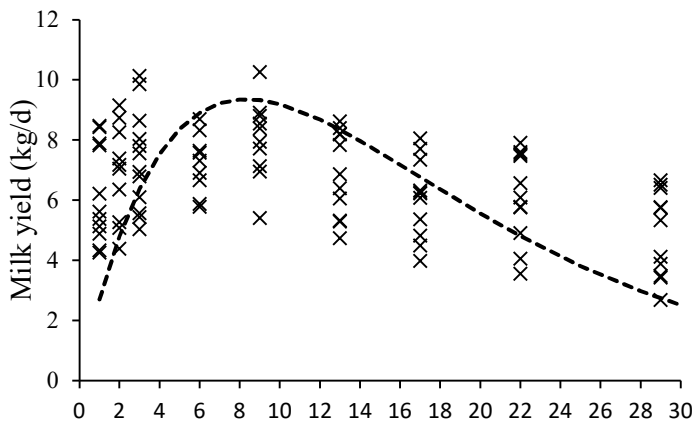
[WD] Wood (1967)

$$y = 6.507 \times (\text{week}^{0.183}) \times e^{(-0.0307 \times \text{week})}$$



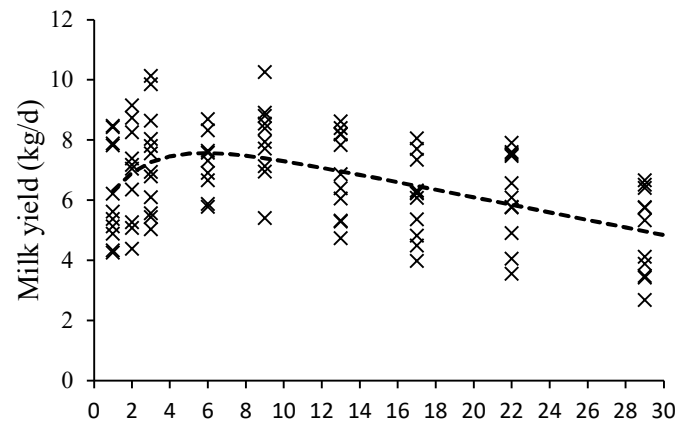
[JF] Jenkins and Ferrell (1984)

$$y = 3.047 \times \text{week} \times e^{(-0.1198 \times \text{week})}$$



[WK] Wilmink (1987)

$$y = 8.614 - 3.240 \times e^{(-0.401 \times \text{week})} - 0.126 \times \text{week}$$



[HR] Henriques et al. (2011)

$$y = 5.074 + (1.224 \times \text{week}) \times e^{(-0.167 \times \text{week})}$$

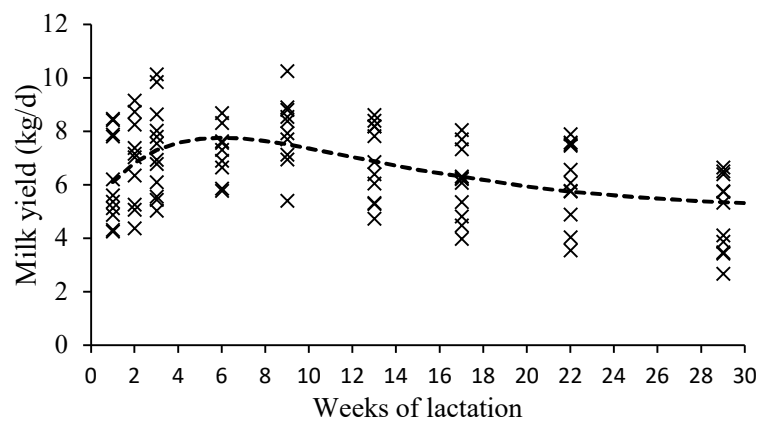
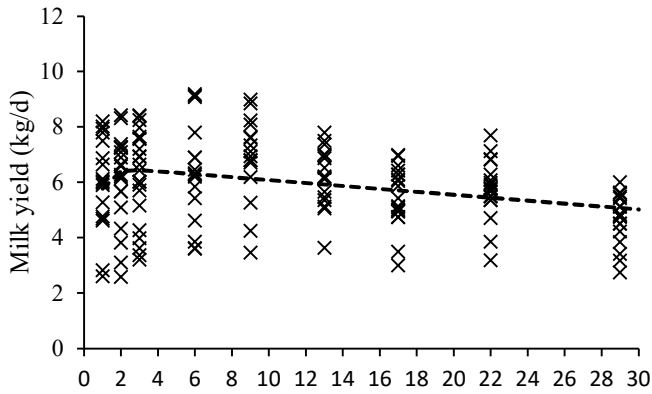


Figure 4. Equations to predict milk yield of Multiparous cows under grazing.

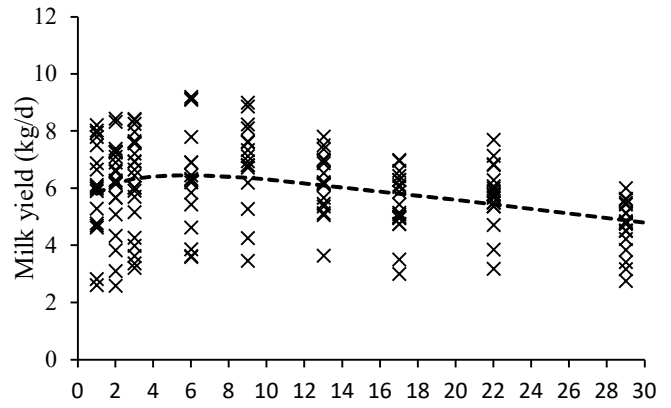
[CL] Cobby and Le Du (1978)

$$y = 6.613 - (0.053 \times \text{week}) - 6.613 \times e^{(-2.129 \times \text{week})}$$



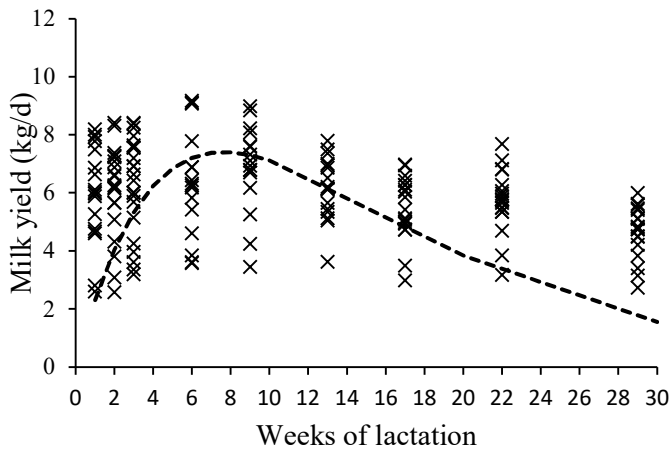
[WD] Wood (1967)

$$y = 5.928 \times (\text{week}^{0.114}) \times e^{(-0.020 \times \text{week})}$$



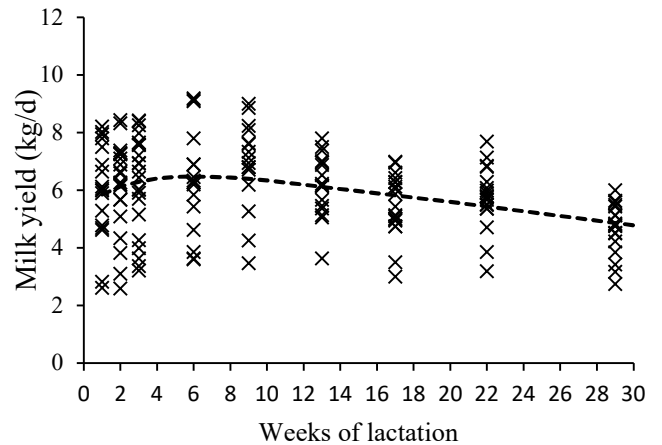
[JF] Jenkins and Ferrell (1984)

$$y = 2.635 \times \text{week} \times e^{(-0.131 \times \text{week})}$$



[WK] Wilmink (1987)

$$y = 7.226 - 1.781 \times e^{(-0.320 \times \text{week})} - 0.0815 \times \text{week}$$



[HR] Henriques et al. (2011)

$$y = 5.060 + (0.754 \times \text{week}) \times e^{(-0.172 \times \text{week})}$$

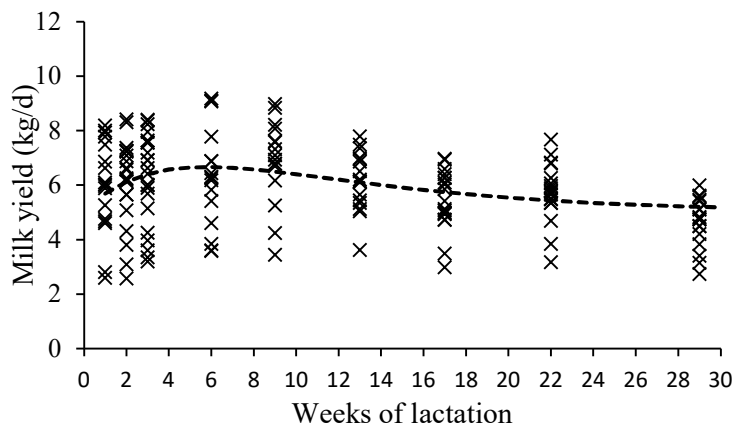


Figure 5. Equations to predict milk yield of nulliparous and primiparous cows under grazing.

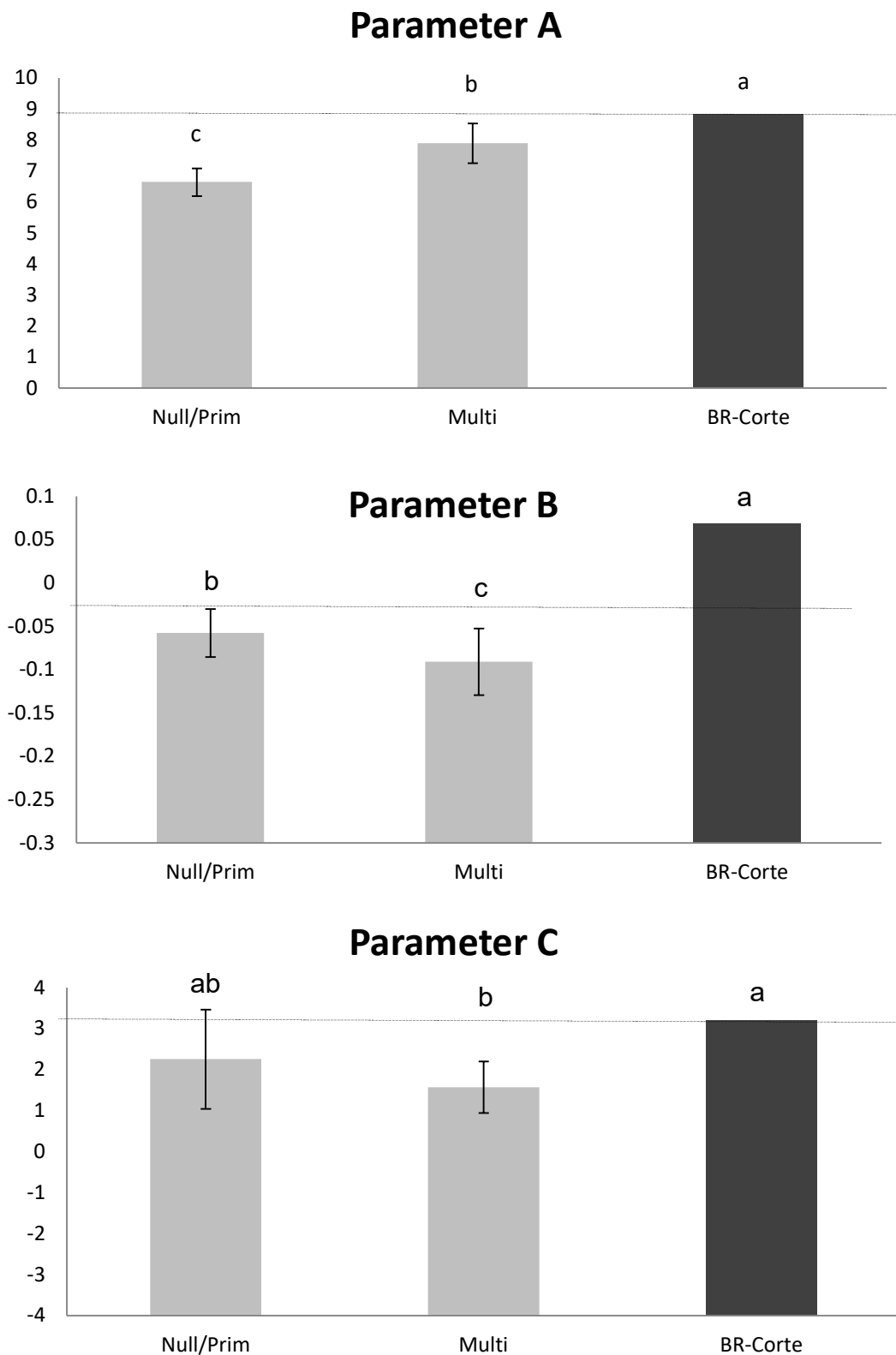


Figure 6. Identity model analysis using a confidence interval approach to compare the parameters ($Y_t = a + bt - e^{-ct}$) of the 3 equations based on CL model: Multi, Null/Prim and BR-Corte. Different letters represent significant differences between parameters values

CHAPTER 3 - Dry matter intake and nutrient digestibility of Nellore cows receiving chopped sugarcane or *Brachiaria decumbens* grass during transition period on feedlot.

Abstract

The study aimed to measure dry matter intake and digestibility of Nellore cows fed chopped sugarcane or *Brachiaria decumbens* grass in the feedlot during the peripartum. Ten pregnant multiparous Nellore cows, with average body weight (BW) of 500±30 was used in this experiment. Cows were randomly divided into two treatments: *ad libitum* supply of sugarcane and *B. decumbens* grass. The sugarcane protein content was corrected with urea to reach the protein level of the *B. decumbens* Cows received a 35% CP supplement during the pre-partum phase in the amount of 1 kg/animal/d and mineral mixed (MM) *ad libitum*. Cows were fed twice a day (7:00h and 15:00h) and intake was adjusted to allow minimal orts without DMI (dry matter intake) restriction. Intake was calculated by weighing the offered feeds and collected orts. The intake measurements started from 24 days before the expected calving date until 24 days postpartum. To evaluate nutrient intake and digestibility, two trials were run: on 14 days before the estimated parturition date and 14 days after parturition. The DMI was similar during pre-partum days except for the parturition that had a decrease of 30% of DMI ($P<0.05$). During pre-partum, the CP and digestible OM intake were higher ($P<0.05$) for the sugarcane cows, but apNDF and iNDF intake were higher for *B. decumbens* cows ($P<0.05$). An interaction occurred between treatments and day for DMI, in which *B. decumbens* cows had higher DMI than sugarcane cows after 17 days post-partum ($P<0.05$). Effect of day was also found, where *B. decumbens* cows started to increase DMI after 17 days in kg per day and in % of BW. Total DM, OM, CP, apNDF, and iNDF intake were higher for *B. decumbens* cows during postpartum. Digestibility of DM, OM, and CP was higher for sugarcane cows ($P<0.05$) at the pre-partum and postpartum period. In summary, the DMI during pre-partum did no change regardless the roughage source, except for a severe decrease at parturition day. The DMI started to increase after 17 days post-partum for the *B. decumbens* treatment.

Keywords: periparturient; *Bos indicus*; digestibility; dry matter intake

Introduction

Understanding the dry matter intake capacity of ruminants (DMI), and how the physiological state affects the magnitude of changes in this sense has great importance for the correct nutritional and reproductive management. Voluntary intake is controlled by environmental and physiological mechanisms that act synergistically to express animals ingestive behavior (Forbes, 1996).

The dry matter intake (DMI) quantification in grazing animals is often difficult because it cannot be measured directly. Thus, the use of internal and external markers is commonly used to estimate forage intake and fecal excretion to measure digestibility (Detmann et al., 2001; Titgemeyer et al., 2001). Moreover, intake and nutrient digestibility assays last from eight to nine days of duration, which involves the application of external markers, that might be inserted by a metal probe via the esophagus, and feces collection. Such intense handling makes the activity stressful, mainly when dealing with pregnant cows in a grazing system.

Several studies have been carried out to assess intake and nutrient digestibility in grazing cows (Ferreira, et al., 2020; Cardenas, 2017; Silva et al., 2017); however, measurements of these parameters for beef cows during transition period have not been made, due to laborious and stressful conditions of handling procedures previous described. In addition, this quantification is punctual, which means that the intake is measured in a short-term period.

Based on this rationale, measuring intake and nutrient digestibility during the transition period for beef cows could be assessed in feedlot systems by harvesting the forage and then offering it to the animal (Rymer, 2000). However, it is necessary to provide a forage source that approximates grazing conditions. The *B. decumbens* is the main forage used in Brazilian grazing systems while chopped sugarcane is an alternative roughage source due to its nutritional value similarities to tropical forages.

Therefore, an experiment was conducted aiming to evaluate the dry matter intake and nutrient digestibility of Nellore cows during the peripartum fed chopped sugarcane or *B. decumbens* grass in feedlot.

Material and Methods

The study was carried out at the Experimental Feedlot facilities of the Department of animal Science at the Federal University of Viçosa (UFV). All animal care and handling

procedures were approved by the Animal Care and Use Committee of the UFV, Brazil (protocol CEUAP-UFV 119/18).

Ten pregnant multiparous Nellore cows, with average body weight (BW) of 500 ± 30 kg was used in this experiment. Cows were taken to the feedlot at 8 months of gestation and placed in individual pens with a concrete floor with a total area of 30 m^2 , where 8 m^2 of the area was undercover, provided with a concrete feeder and trough for water.

Cows were randomly divided into two treatments: *ad libitum* supply of sugarcane and *Brachiaria decumbens* grass. Sugarcane (variety RB-867515) was harvested daily in the afternoon and chopped before the morning feeding. The *B. decumbens* grass was cut 10 cm from the soil three times a week with a brush cutter attached to a tractor. As recommended by Minson (1981), forage was cut in the afternoon, and sufficient forage for two days until the next harvest. Cutting in the afternoon avoids contamination of the forage with morning dew. The forage was stored overnight and kept refrigerated in a cold chamber to minimize losses and prevent fermentation (Minson, 1981). The *B. decumbens* was supplied in whole form to the animals to allow selection.

The sugarcane protein content was corrected with urea to reach the protein level of the *B. decumbens* to adjust the ratio of urea supplied. Before the beginning of the study and after parturition date, a representative sample of sugarcane and *B. decumbens* were collected at the area used for harvest and quantified for crude protein (CP) and dry matter (DM). The information of the samples analyzed at the beginning of the experiment was used for the whole pre-calving, and the samples collected during parturition period was used for post-partum. Thereafter, sugarcane DM content was quantified weekly.

All cows received a 35% CP supplement during the pre-partum phase in the amount of 1 kg/animal/d and they had free access to mineral mix (MM) (41.2% - corn meal, 36.0% - soybean meal, 20.0% - wheat bran and 2.8% - urea: ammonium sulfate). Supplement was supplied in additional feeders. After calving, cows remained in the same pens but received only MM until 24 days on lactation. The supplementation was supplied only during pre-partum since it is a recommended nutritional management for beef cows during this phase.

Cows were fed twice a day (7:00h and 15:00h) and feed intake was adjusted to allow minimal orts without DMI restriction. Feed intake was calculated by weighing the offered feeds and collected orts. Diets were offered on an *ad libitum* basis (target 100 g refusal/kg fed) and the amount offered was adjusted daily. The method adopted to adjust minimal orts included a visual analysis, which consisted in identifying edible orts, to make sure that the animal could express a real *ad libitum* DMI.

The amount of feed supplied was recorded daily; additionally, the ingredients in the concentrate were sampled at every concentrate manufacturing time. Sugarcane and *B. decumbens* were sampled every day, stored, and a composite sample was created over 7 d period.

The cows had 10 days of adaptation to the conditions of the feedlot environment, and intake measurements started from 24 days before the expected calving date until 24 days postpartum. The calves were kept in cow pens throughout the experiment. Cows were weighed at the beginning of the experiment (-24 days pre-partum), and 7 days before the expected calving day and at the end of the experiment (24 days).

To evaluate nutrient intake and digestibility, an assay was conducted on day 14 before the estimated parturition date (around 276 days of gestation) and 14 days after parturition. Indigestible neutral detergent fiber (iNDF) was used to estimate the fecal excretion of animals. Fecal samples were collected immediately after defecation during four days (one sample per day) at 18:00h, 14:00h, 10:00h, and 06:00h. Feces samples were partially dried at 55°C for 72 hours and ground to pass through 1- and 2-mm screens in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA). Then, a 100-g composite sample was built collecting 25 g from each feces collection time.

Sugarcane, *B. decumbens*, feces, and supplement samples were analyzed following the procedures described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA) (Detmann et al., 2012) for DM (index INCT-CA method G-003/1), ash (index INCT-CA method M-001/1), CP (index INCT-CA method N-001/1), neutral detergent fiber corrected for ash and protein (apNDF; index INCT-CA method F-002/1). Indigestible neutral detergent fiber (iNDF) was processed at 2 mm and quantified by *in situ* incubation procedures with nonwoven textile bags (100 g/m²) for 288 h (Valente et al., 2011).

Chemical composition of the energy-protein supplement, sugarcane and *B. decumbens* provided to cows during pre- and postpartum are shown in table 1.

Statistical analyses

Analyses of variance (ANOVA) for the nutrient digestibility variables measured during pre- and postpartum were performed using the following model:

$$Y_{ij} = \mu + T_i + e_{(i)j}$$

where: Y_{ij} = observation took on animal j , submitted to treatment i ; μ = overall constant; T_i = fixed effect of the treatment i ; $e_{(i)j}$ = random effect assumed to be NIID (0, σ^2_e). The

measurements of DMI pre- and post-partum were analyzed as repeated measures over time. Effects of treatment, day and treatment x day interaction were analyzed.

All the statistical evaluations were performed considering 0.05 as the critical level of probability for the occurrence of the type I error. The statistical analyses were carried out using the PROC MIXED of SAS 9.4 (Inst. Inc., Cary, NC, USA).

Results and discussion

Intake and digestibility pre-partum

The DMI was not different among treatments during pre-partum ($P>0.05$) but it had effect of day ($P<0.05$). DMI was similar during pre-partum days except for the parturition that had a decrease of 30% of DMI ($P<0.05$; Figure 1a and 1b).

It's well known in dairy research that cows have an average reduction of 10 to 30% of DMI during the last 21 days pre-partum (NRC, 2001; Drackley, 1998). The dry matter intake decreases about 32% during the final three weeks of gestation, and 89% of that decline occurs at five to seven days before calving (Hayirli et al., 2002). The present experiment shows that the DMI for Nellore cows during the last 24 days before parturition did not change, only decreasing at calving, which indicates that possibly, the reduction in DMI for Nellore cows starts before 24 days, regardless of the roughage source. Although the DMI did not change at the pre-partum period, intake was around 28% lower than the suggested by the BR-Corte (2016) for pregnant cows at 270 days of gestation.

In grazing conditions, Silva et al., (2017) and Ferreira et al., (2020) found an intake average of 10 and 16.25 g/kg BW of DMI, respectively, at 45 days before parturition in multiparous cows. Cardenas (2017) obtained a value of 16.20 g/kg BW of DMI at 30 days before parturition, and an average of 20 g/kg at 20 days postpartum. The BR-Corte (2016) proposed an equation to estimate DMI of pregnant cows, and an equation to estimate DMI during lactation with the addition of an adjustment for average daily gain. However, there are still limited data, as only a few experiments have assessed nutrient intake and digestibility of Nellore cows in the early lactation (Valente et al., 2013; Costa e Silva, 2015) and pre-calving period (Gionbelli, 2013).

Based on literature data and herein presented, is possible that a more several DMI reduction of Nellore cows starts between 30 to 21 days before calving, since DMI at 30 and 45 days from Cardenas (2017) and Ferreira et al., (2020) were extremely similar (1.6 and 1.5

%BW, respectively), but much higher than DMI observed in this study at 21 days (1.2 % BW).

The DMI regulation has been associated with physical and physiological factors. Basically, some theories are based on the physical fulfill of the reticulorumen (Allen, 1996; Mertens, 1994) or metabolic-feedback factors (Illius and Jessop, 1996; Mertens, 1994). However, during parturition and transition period, other physiological effects influence the DMI reduction, such as the effect of fetal growth at late gestation which reduces reticulorumen space and thus the capability of feed storage (Forbes, 1968), as well as estrogens feedback at late gestation and early lactation (Forbes, 2007). Parturition is also an event of extreme stress conditions, so the DMI reduction could be also related to heightened stress hormones and endorphins during this period (Forbes, 2007). There are many theories that explains to reduction of DMI, however each theory might be applicable under different conditions and physiological state of the animal, thus, it is most likely that the DMI regulation is an additive effect of several stimuli (Forbes, 1996).

The intakes of total DM and OM were not different between treatments, even when expressed in g/kg BW. The CP and digestible OM intake were higher ($P < 0.05$) for the cows fed sugarcane, but apNDF and iNDF intake were higher for the cows fed *Brachiaria d.* ($P < 0.05$; Table 2). The dNDF was not different among treatments ($P > 0.05$).

Digestibility of DM, OM, and CP was higher for sugarcane cows ($P < 0.05$), however, the apNDF digestibility were similar between treatments ($P > 0.05$; Table 2).

We hypothesized that, as cows approach parturition day, nutrient digestibility would reduce due to a possible heightened passage rate that would happen because of a reduction of ruminal space (Forbes, 2007). Available digestibility data on the literature in similar feed conditions of this experiment are all before 14 days pre-partum. The values for *B. decumbens* nutrient digestibility at the end of gestation herein found are fairly in agreement with other experiments, even collected at different times of gestation (Cardenas, 2017; Silva et al., 2017; Ferreira et al., 2020).

Sugarcane digestibility in general, except for apNDF, was higher than *B. decumbens* possibly due to the CP correction with urea that increased CP digestibility since urea was the major part of CP content of the diet, which led to a higher OM digestibility.

Intake and digestibility postpartum

An interaction occurred between treatments and day for DMI, in which *B. decumbens* cows started to increase DMI after 17 days in kg per day and in % of BW, while sugarcane did no change ($P < 0.05$; Figure 2a).

Early post-partum DMI did not change until 17 days, where it increases linearly reaching approximately 2% of the body weight at day 24 (Figure 2b). Only few experiments have evaluated intake and digestibility during post-partum of grazing beef cows. Cardenas (2017) at 25 days after parturition found a DMI of around 10.5 kg which represents 2% of BW. Costa e Silva (2015) and Lopes (2015) found 2.5 and 2.4 %BW DMI from cows at 28d and 30d postpartum, respectively. This in addition to our results, strongly suggest that the beginning of return and stabilization of DMI starts around 25 to 30 days postpartum. This is also in agreement with dairy cows, that shows a rapidly increase DMI for the first 21 days after calving (Ingvarlsen and Andersen, 2000).

Digestibility of DM, OM, CP, and apNDF was higher for sugarcane cows ($P < 0.05$), for the same reason mentioned about the digestibility assay at the pre-partum period. Although sugarcane displayed higher nutrient digestibility, total DM, OM, CP, apNDF, and iNDF intake were higher for *B. decumbens* cows, expressed in kg/d and g/kg body weight ($P < 0.05$; Table 3). The dOM and dNDF were not different between treatments ($P > 0.05$).

B. decumbens digestibility post-partum is in accordance with literature data (Cardenas, 2017, Lopes, 2015), except for CP digestibility, due to the difference of forage quality between the studies.

Conclusion

The DMI during pre-partum did no change regardless the roughage source, except for a severe decrease at parturition day. The DMI started to increase after 17 days post-partum for the *B. decumbens* treatment.

Table 1. Chemical composition of supplement, sugarcane and *B. decumbens* provided to cows during pre- and postpartum.

Item ¹	Suppl ²	Sugarcane	<i>B. decumbens</i>
<i>Pre-partum</i> ²			
MS	893	252	341
OM	952	974	869
CP	364	28.8	57.1
NDF	143	551	703
apNDF	136	542	626
iNDF	17.1	248	279
<i>Postpartum</i> ²			
MS	-	262	282
OM	-	973	893
CP	-	25.2	49.2
NDF	-	542	720
apNDF	-	526	653
iNDF	-	215	245

¹dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), neutral detergent fiber corrected for ash and protein (apNDF), indigestible NDF (iNDF).

¹g/kg of DM. ² Energy-protein supplement (Suppl).

²intake and digestibility assay.

Table 2. Intake and digestibility of nutrients from Nellore cows receiving chopped sugarcane or *B. decumbens* at 14d pre-calving.

Items	Treatments		SEM	P-value
	Sugarcane	<i>B. decumbens</i>		
	<i>intake, kg/d</i>			
DM	5.93	6.56	0.273	0.141
OM	5.77	6.39	0.265	0.135
CP	0.59	0.42	0.012	<.0001
apNDF	2.80	3.67	0.160	0.005
iNDF	1.23	1.89	0.076	0.003
dOM	3.87	3.24	0.139	0.012
dNDF	3.04	3.19	0.144	0.480
	<i>intake, g/kg, body weight</i>			
DM	10.08	12.61	0.681	0.106
OM	10.52	12.29	0.667	0.098
apNDF	5.11	7.05	0.539	0.008
iNDF	2.25	3.64	0.191	0.009
	<i>nutrient digestibility, %</i>			
DM	65.50	44.66	1.10	<0.000
OM	65.40	49.60	1.11	<0.001
CP	73.60	39.90	1.54	<0.001
apNDF	51.24	48.86	1.33	0.242

Total dry matter intake (DM), dry matter of forage intake (DMF), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (apNDF), indigestible NDF (iNDF), digested organic matter (dOM), digestible NDF (dNDF).

Table 3. Intake and digestibility of nutrients from Nellore cows receiving chopped sugarcane or *B. decumbens* at 14d postpartum.

Items	Treatments		SEM	<i>P</i> -value
	Sugarcane	<i>B. decumbens</i>		
	<i>intake, kg/d</i>			
DM	5.37	6.89	0.329	0.010
OM	5.24	6.72	0.318	0.010
CP	0.24	0.34	0.014	0.001
apNDF	2.82	3.62	0.172	0.001
iNDF	1.09	1.43	0.078	0.015
dOM	3.69	3.80	0.21	0.706
dNDF	2.97	2.88	0.20	0.751
	<i>intake g/kg, body weight</i>			
DM	10.64	15.27	0.788	0.003
OM	10.39	14.89	0.767	0.003
apNDF	5.59	8.03	0.415	0.003
iNDF	2.16	3.17	0.173	0.003
	<i>nutrient digestibility, %</i>			
DM	66.60	48.06	1.75	<0.001
OM	68.68	55.20	1.32	<0.001
CP	49.80	35.22	5.32	0.08
apNDF	55.30	41.80	1.59	<0.001

Total dry matter intake (DM), dry matter of forage intake (DMF), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (apNDF), indigestible NDF (iNDF), digested organic matter (dOM), digestible NDF (dNDF).

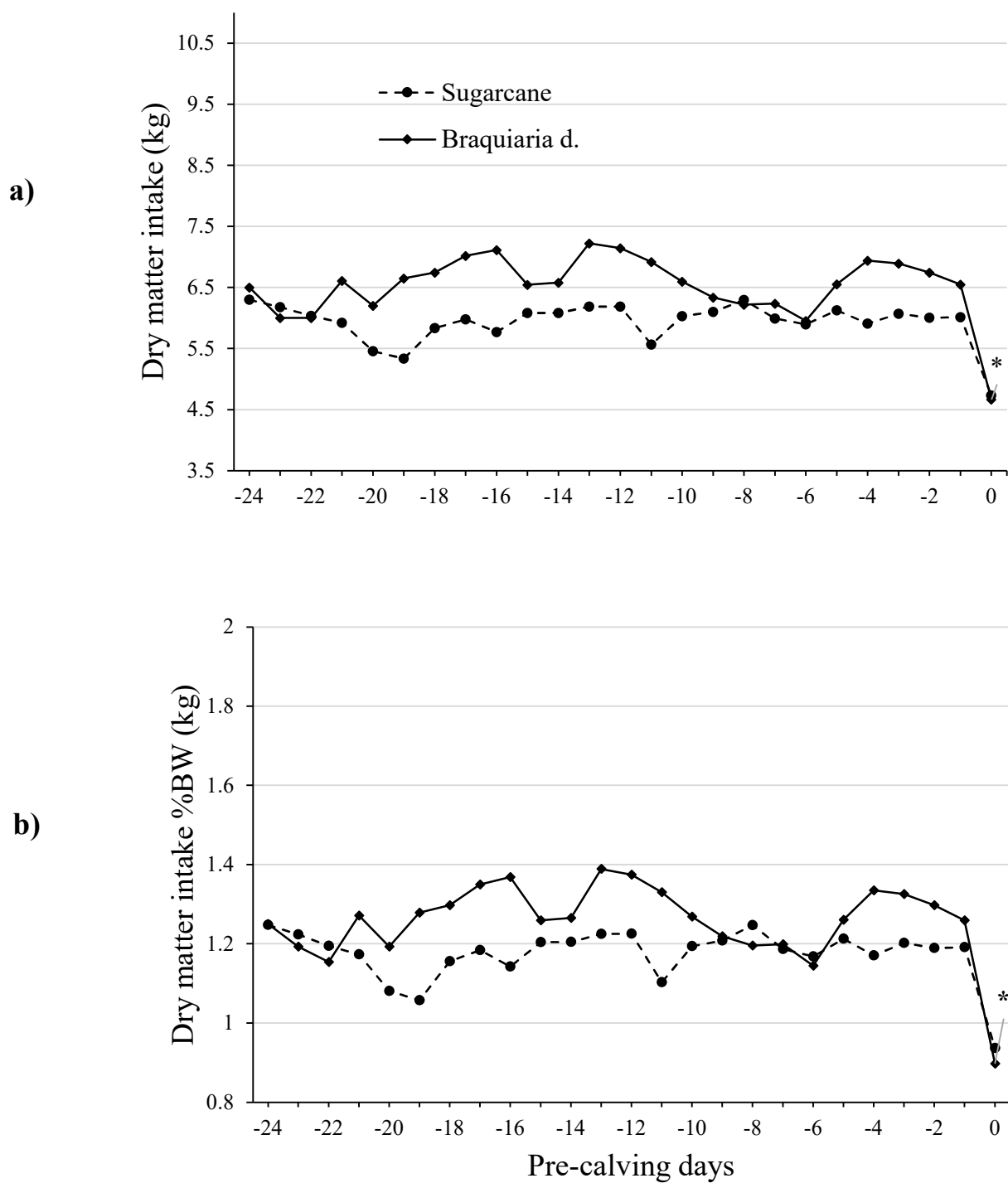


Figure 1. Dry matter intake (kg and % BW) of Nellore cows receiving sugar-cane and *B. decumbens* as roughage source during pre-partum. Asterisks (*) declare significant difference between days at $P < 0.05$.

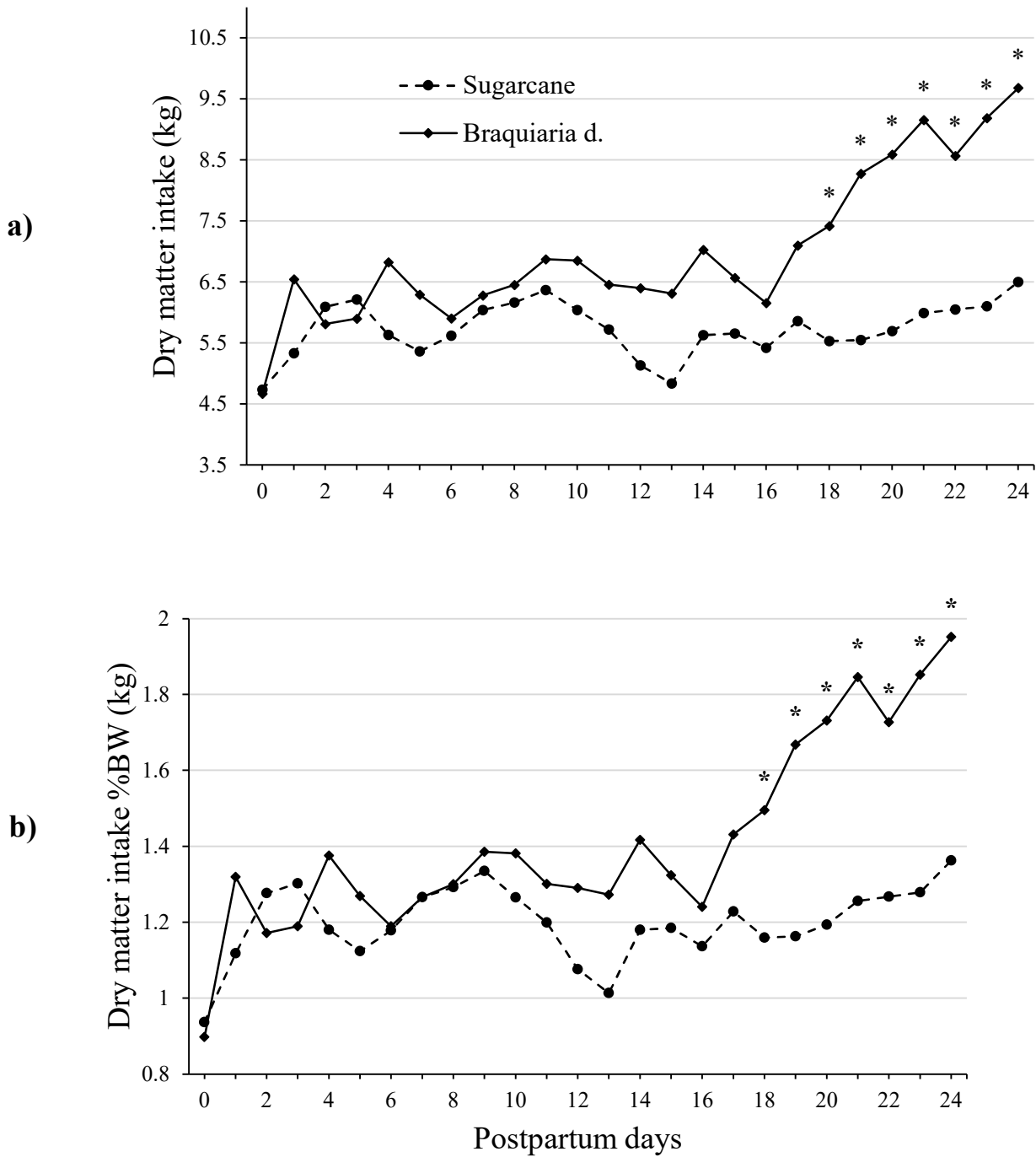


Figure 2. Dry matter intake (kg and % BW) of Nellore cows receiving sugar-cane and *B. decumbens* as roughage source during postpartum. Asterisks (*) declare significant difference between treatments and between days at $P < 0.05$.

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