**CAMILA DE PAULA**

# PARITY IN COW-CALF PAIR: STRESS RESPONSE AT WEANING AND GROWTH CHARACTERISTICS OF NELLORE HEIFERS ON PRE- AND POST-WEANING

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
Co-adviser: Sebastião de Campos Valadares Filho

**VIÇOSA - MINAS GERAIS**
**2023**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa**

T

P324p
2023

Paula, Camila de, 1992-
Parity in cow-calf pair: stress response at weaning and growth characteristics / Camila de Paula. – Viçosa, MG, 2023.
1 tese eletrônica (89 f.): il.

Texto em inglês.
Orientador: Luciana Navajas Rennó.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2023.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2023.394
Modo de acesso: World Wide Web.

1. Bovinos de leite. 2. Bezerros. 3. Desmame - Efeito fisiológico. I. Rennó, Luciana Navajas, 1973-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.2142

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

**CAMILA DE PAULA**

# PARITY IN COW-CALF PAIR: STRESS RESPONSE AT WEANING AND GROWTH CHARACTERISTICS OF NELLORE HEIFERS ON PRE- AND POST-WEANING

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*.

APROVED: June 16, 2023

Assent:

Camila de Paula
Author

Luciana Navajas Rennó
Adviser

*To my parents João (in memorian) and Nailza, my brother Marcelo, my nephew Lucas, my niece Izabella, my fiancé Cleiton and mother-in-law Aparecida for all the love, care, encouragement, and support.*

**I dedicate**

# ACKNOWLEDGEMENTS

I would like to thank God, for the strength and for always lighting my way, moreover, for providing special moments and people.

The journey has been long and therefore, I am especially grateful to my family who have been always by my side. Thank you for believing in me!

Especially to my fiancé Cleiton Magalhães, who was always there.

To the Universidade Federal de Viçosa, especially to the Department of Animal Science, for the opportunity to carry out this work and to all employees who, in some way, are part of our day-to-day activities.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting the scholarship.

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA) for providing resources to carry out this work.

To my adviser, Professor Luciana Navajas Rennó, for all the moments, teachings, guidance, affection, and patience of a "mother", certainly without you none of this would be possible.

To all the professors of the Animal Science Department, in particular, the professors Mario Paulino, Edenio Detmann, Sebastião de Campos Valadares Filho, and Giancarlo Magalhães dos Santos for all their support, patience, and advice.

To all my friends of the DZO, postgraduates, interns, and friends from Animal Laboratory, Laboratory of Meat Science, and UEPE - GL. Especially to Matheus Fellipe de Lana Ferreira who had great merit in this study.

To the employees of the UEPE - Beef Cattle (Neco, Norival, and Marcelino), Animal Nutrition Laboratory, Animal Physiology, and Animal Laboratory for their help in carrying out the fieldwork, collaboration in laboratory analysis, and companionship.

Thank you to everyone who has somehow been by my side. This achievement is also yours!!!

*"Compassion for animals is intimately connected with goodness of character, and it may be safely asserted that he who is cruel to animals cannot be a good man."*

(Arthur Schopenhauer)

**BIOGRAPHY**

CAMILA DE PAULA, daughter of João Aniceto de Paula (in memoriam) and Nailza do Prado, was born in Guarulhos, São Paulo, on April 12 of 1992.

She joined the Animal science undergrad at Universidade Federal de Viçosa in March 2010 and received her bachelor's degree in January 2015.

She started her master's degree at Universidade Federal de Viçosa in March 2017, with a major in Animal Physiology and Production, concluding this course in 2020. Then, she started her Ph.D. course in the same area and department, concluding the thesis in June 2023.

# ABSTRACT

DE PAULA, Camila, D.Sc., Universidade Federal de Viçosa, June, 2023. **Parity in cow-calf pair: stress response at weaning and growth characteristics of Nellore heifers on pre- and post-weaning**. Adviser: Luciana Navajas Rennó. Co-adviser: Sebastião de Campos Valadares Filho.

The cow's milk yield has been described as a factor that influences the performance of calves in the pre-and post-weaning phases. Furthermore, weaning is known to generate multiple stressors in the calf-cow pair. Nonetheless, little is known whether these responses differ according to parity order. The goal of these three studies was to evaluate whether calving order influences the weaning stress response in the cow-calf pair and the effects of dam's parity in pre- and post-weaning beef heifers: performance, growth, metabolism, hormonal and reproductive characteristics. Thirty pregnant Nellore cows with their respective female Nellore calves were randomly allocated to five paddocks and two cow-calf pairs from each parity group were placed in the paddocks. The main results of the first study, there was an interaction ($P < 0.05$) between parity and evaluation days regarding cortisol, where on d +7, the higher concentration was observed for multiparous cows. There was an interaction ($P < 0.05$) between parity and evaluation day for red blood cells (RBC), hematocrit (HCT), and hemoglobin (HB), whereby higher RBC counts on d +4 were observed for multiparous cows. For HCT and HB, on all post-weaning collection days, higher values were observed for multiparous cows. The concentrations of ceruloplasmin, haptoglobin, total proteins, albumin, and the number of white blood cells, neutrophils and lymphocytes varied over the days of collection ($P < 0.05$). The day of evaluation had an ($P < 0.05$) effect on all recorded behaviors, except for rumination ($P > 0.05$). In the second study, the initial body weight (BW), final BW, and average daily gain (ADG) of heifers were similar between parities ($P > 0.05$), in the pre-weaning phase. In response to weaning stress, BW was not affected by the interaction ($P > 0.05$), however, it was affected by day ($P < 0.05$). Only the cortisol concentration of the heifers was influenced by the dam's parity ($P < 0.05$), with a higher concentration for heifers born to multiparous than primiparous cows. For most of the physiological measurements, there was variation over the days of collection ($P < 0.05$), except for haptoglobin concentration ($P > 0.05$) and neutrophils: lymphocytes ratio ($P > 0.05$). In the third study, the main results found in the post-weaning were that initial and final BW and ADG of heifers were similar between treatments ($P > 0.05$). Body measurements were similar between heifers ($P > 0.05$), except that rump length was greater for heifers from multiparous than primiparous cows ($P < 0.05$). In the dry season (d 84), the subcutaneous fat thickness of the loin and subcutaneous fat thickness on the rump were higher for heifers from multiparous cows when compared to heifers from secundiparous cows ($P < 0.05$). At the end of the experiment, all body composition measurements were similar between parities ($P > 0.05$). In all measurements studied, the metabolic profile of heifers was similar between parities ($P > 0.05$). Except, on d 84, triglycerides and very-low density lipoprotein were higher for heifers from primiparous than heifers from secundiparous and multiparous cows ($P < 0.05$). Also, progesterone, leptin, and Insulin-like growth factor 1 concentrations were similar among heifers ($P > 0.05$). In addition, the dam's parity did not influence reproductive performance ($P > 0.05$). Nellore cows, regardless of parity, undergo behavioral and physiological changes in abrupt weaning. Parity influences physiological parameters indicating that the magnitude of stress is greater in multiparous cows. In Nellore heifers, there are physiological changes caused by abrupt weaning, but they are slight influenced by the parity of the dams. In addition, in the post-weaning period, maternal parity is a factor that has slight influence on the performance, metabolic, hormonal, and reproductive characteristics of Nellore heifers on pasture.

Keywords: *Bos indicus*. Maternal effects. Physiological changes. Behavior. Growth rate. Puberty.

**RESUMO**

DE PAULA, Camila, D.Sc., Universidade Federal de Viçosa, junho de 2023. **Parity in cow-calf pair: stress response at weaning and growth characteristics of Nellore heifers on pre- and post-weaning**. Orientadora: Luciana Navajas Rennó. Coorientador: Sebastião de Campos Valadares Filho.

A produção de leite da vaca tem sido descrita como um fator que influencia o desempenho de bezerros nas fases pré e pós-desmame. Além disso, sabe-se que o desmame gera múltiplos estressores no par bezerro-vaca. No entanto, pouco se sabe se essas respostas diferem de acordo com a ordem de paridade. O objetivo desses três estudos foi avaliar se a ordem de parto influencia a resposta ao estresse no desmame no par vaca-bezerro e os efeitos da ordem de parto da mãe sob as novilhas de corte nos períodos pré e pós-desmame: desempenho, crescimento, metabolismo, hormonal e reprodutivo. Trinta vacas Nelore prenhas com suas respectivas bezerras Nelore foram alocadas aleatoriamente em cinco piquetes e dois pares bezerras-vacas de cada grupo de paridade foram alocados nos piquetes. Como principais resultados do primeiro estudo, houve uma interação ($P < 0,05$) entre os dias e paridade para a concentrações de cortisol, onde no dia +7, a maior concentração foi observada para vacas multíparas. Houve interação ($P < 0,05$) entre a paridade e o dia da avaliação para hemácias (RBC), hematócrito (HCT) e hemoglobina (HB), sendo observadas maiores contagens de hemácias no dia +4 para vacas multíparas. Para HCT e HB, em todos os dias de coleta pós-desmame, valores maiores foram observados para vacas multíparas. As concentrações de ceruloplasmina, haptoglobina, proteínas totais, albumina e o número de leucócitos, neutrófilos e linfócitos variaram ao longo dos dias de coleta ($P < 0.05$). O dia da avaliação teve efeito ($P < 0,05$) em todos os comportamentos registrados, exceto ruminação ($P > 0,05$). No segundo estudo, na fase de pré-desmame, o peso corporal inicial (PC), o PC final e o ganho médio diário (GMD) das novilhas foram semelhantes entre os tratamentos ($P > 0,05$). Em resposta ao estresse do desmame, o PC não foi afetado pela interação ($P > 0,05$), porém foi afetado pelo dia ($P < 0,05$). Apenas a concentração de cortisol das novilhas foi influenciada pela paridade da mãe ($P < 0,05$), com maior concentração para novilhas nascidas de vacas multíparas quando comparas com as filhas de vacas primíparas. Para a maioria das medidas fisiológicas, houve variação ao longo dos dias de coleta ($P < 0,05$), exceto para a concentração de haptoglobina ($P > 0,05$) e relação neutrófilos: linfócitos ($P > 0,05$). No terceiro estudo, os principais resultados encontrados na fase de pós-desmame foram que o PC inicial e final e o GMD das novilhas foram semelhantes entre os tratamentos ($P > 0,05$). As medidas corporais foram semelhantes entre as novilhas (P

> 0,05), exceto que o comprimento da garupa foi maior nas novilhas filhas de vacas multíparas quando comparadas com as filhas das vacas primíparas (P < 0,05). No período de seca (d 84), a espessura de gordura subcutânea do lombo e na garupa foi maior para novilhas filhas de vacas multíparas quando comparadas com novilhas filhas de vacas secundíparas (P < 0,05). Ao final do experimento, todas as medidas de composição corporal foram semelhantes entre as novilhas (P > 0,05). Em todas as medições estudadas, o perfil metabólico das novilhas foi semelhante entre os tratamentos (P > 0,05). Exceto, no dia 84, triglicerídeos e lipoproteína de baixa densidade foram maiores para novilhas filhas de vacas primíparas do que novilhas filhas de vacas secundíparas e multíparas (P < 0,05). Além disso, as concentrações de progesterona, leptina e fator de crescimento semelhante à insulina 1 foram semelhantes entre as novilhas (P > 0,05). As vacas Nelore, independentemente da ordem de parto, sofrem alterações comportamentais e fisiológicas no desmame abrupto. A paridade influencia os parâmetros fisiológicos indicando que a magnitude do estresse é maior em vacas pluríparas. Em novilhas Nelore, ocorrem alterações fisiológicas causadas pelo desmame abrupto, mas estas são pouco influenciadas pela paridade das mães. Além disso, no período pós-desmame, a paridade materna é um fator que pouco influencia no desempenho, nas características metabólicas, hormonais e reprodutivas de novilhas Nelore a pasto.

Palavras-chave: *Bos indicus*. Efeitos maternos. Alterações fisiológicas. Comportamento. Taxa de crescimento. Puberdade.

## SUMMARY

# GENERAL INTRODUCTION

Decreasing the age at puberty of heifers allows for greater precocity and selection pressure in choosing cows with better efficiency. In this way, cows will be able to produce more calves in their productive life with a faster return on investment (Vaz *et al.* 2012). However, when the production system is intensified, reducing the age at first calving to 24 months, becomes necessary to improve the productive and reproductive performance of the cow and the calf. In this context, weaning is a necessary and indispensable practice, however, it is known to generate multiple stressors in the cow-calf pair.

Whereas in nature weaning is a gradual process, which occurs as maternal care and milk yields are reduced, in the beef cattle production system, weaning is usually carried out abruptly and around seven months of age. This means that the calf must adapt simultaneously to multiple stressors, which cause especially acute and prolonged effects, such as the loss of the mother, changes in diet, and the physical and social environment (Ungerfeld *et al.* 2015; Lynch *et al.* 2019; Orihuela and Galina, 2019).

As a result, a series of actions in the cow-calf pair is triggered in response to weaning, such as increased vocalization and locomotion, reduced grazing and rumination time (Ungerfeld *et al.* 2011, 2015; Pérez-Torres *et al.* 2016, 2021; Stěhulová *et al.* 2017). In addition, it can cause a series of physiological responses, such as an increase in the concentration of cortisol, acute phase proteins, and white blood cell counts that which is directly related to depression in the immune system and lower productive performance (Carroll *et al.* 2009; Lynch *et al.* 2011; Campistol *et al.* 2016).

The most important maternal effects that influence calf performance are the uterine environment during pregnancy, adequate colostrum with antibody transfer, maternal ability to protect the calf, and milk yield (Quintanilla and Piedrafita 2000). Thus, it is described that the parity order affects the birth weight, weaning weight, and maturity of the animals, being verified, lower weight at birth, weaning, and maturity in calves offspring of cows with one or two calves (Cortés-Lacruz *et al.* 2017). These results are justified by the authors, due to a lower maternal ability, milk yield, and, consequently, lower intake by the calf.

Differences observed in maternal ability can be related to the mother's temperament and to the reproductive experience, so that female mammals in general are better mothers after the second birth (Paranhos da Costa *et al.* 2007). Indeed, lower milk yields are reported in primiparous cows than in multiparous cows, this result may be caused by the metabolic differences of these animals since primiparous cows require nutrients to continue their growth (Paape *et al.* 2000; Pimentel *et al.* 2006; Ferreira, Rennó, Rodrigues, Detmann, *et al.* 2021).

Undoubtedly, milk is the main source of nutrients for the calf in the first months of life and the quantity and quality of milk can have implications for the future performance of the calf (Linden *et al.* 2014). Furthermore, cow milk production has been described to influence the behavior of calves abruptly weaning at 6 months (Ungerfeld *et al.* 2009). However, although responses to early weaning are influenced by parity order, being more pronounced in multiparous than in primiparous (Ungerfeld *et al.* 2011), little is known whether these responses are similar in the cow-calf pair conventionally weaned (7 to 8 months).

The post-weaning phase is a critical period since seasonal variations in rainfall and temperature are some of the factors that are responsible for the inferior growth performance, caused by decreased forage quality (Mpofu *et al.* 2022; de Paula *et al.* 2022). Additionally, the nutritional status of the animal directly influences the time until puberty, being a critical point, since hormones such as insulin (Adam and Findlay 2001), insulin-like growth factor (IGF-1) (Hiney *et al.* 1991), leptin, and metabolites such as glucose and free fatty acids (Garcia *et al.* 2003) may act on the hypothalamus to regulate food intake, energy expenditure, and reproduction, mainly by stimulating the production of gonadotropin-releasing hormone (Stanley *et al.* 2005).

Therefore, it is still unclear whether the cow's parity order can influence the intensity of the dam's nutritional and social dependence on the calf, which can lead to changes in the ability to adapt to weaning in the cow-calf pair, and whether it influences the performance, growth, metabolic and hormonal characteristics in Nellore heifers on the pre- and post-weaning.

**REFERENCES**

Adam CL, Findlay PA (2001) Inhibition of Luteinizing Hormone Secretion and Expression of c-fos and Corticotrophin-Releasing Factor Genes in the Paraventricular Nucleus During Insulin-Induced Hypoglycaemia in Sheep: Inhibition of Luteinizing Hormone Secretion and Expression of c-. Journal of Neuroendocrinology 10, 777–784. doi:10.1046/j.1365-2826.1998.00263.x.

Campistol C, Kattesh HG, Waller JC, Rawls EL, Arthington JD, Carroll JA, Pighetti GM, Saxton AM (2016) Effects of pre-weaning feed supplementation and total versus fenceline weaning on the physiology and performance of beef steers. International Journal of Livestock Production 7, 48–54. doi:10.5897/IJLP2016.0291.

Carroll JA, Arthington JD, Chase CC (2009) Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves1. Journal of Animal Science 87, 4167–4172. doi:10.2527/jas.2009-2016.

Cortés-Lacruz X, Casasús I, Revilla R, Sanz A, Blanco M, Villalba D (2017) The milk yield of dams and its relation to direct and maternal genetic components of weaning weight in beef cattle. Livestock Science 202, 143–149. doi:10.1016/j.livsci.2017.05.025.

Ferreira MF de L, Rennó LN, Rodrigues II, Detmann E, Paulino MF, de Campos Valadares Filho S, Martins HC, Moreira SS, de Lana DS (2021) Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods. BMC Veterinary Research 17, 311. doi:10.1186/s12917-021-03019-0.

Garcia MR, Amstalden M, Morrison CD, Keisler DH, Williams GL (2003) Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age1. Journal of Animal Science 81, 261–268. doi:10.2527/2003.811261x.

Linden DR, Titgemeyer EC, Olson KC, Anderson DE (2014) Effects of gestation and lactation on forage intake, digestion, and passage rates of primiparous beef heifers and multiparous beef cows1. Journal of Animal Science 92, 2141–2151. doi:10.2527/jas.2013-6813.

Lynch EM, McGee M, Doyle S, Earley B (2011) Effect of post-weaning management practices on physiological and immunological responses of weaned beef calves. Irish Journal of Agricultural and Food Research 50,.

Lynch E, McGee M, Earley B (2019) Weaning management of beef calves with implications for animal health and welfare. Journal of Applied Animal Research 47, 167–175. doi:10.1080/09712119.2019.1594825.

Mpofu TJ, Nephawe KA, Ginindza MM, Siwendu NA, Mtileni B (2022) Cow Efficiency, Relative-Birth Weight and Subsequent Pre-Weaning Growth Performance of Nguni Cattle. American Journal of Animal and Veterinary Sciences 17, 113–121. doi:10.3844/ajavsp.2022.113.121.

Orihuela A, Galina CS (2019) Effects of Separation of Cows and Calves on Reproductive Performance and Animal Welfare in Tropical Beef Cattle. Animals 9, 223. doi:10.3390/ani9050223.

de Paula C, Rennó LN, Ferreira MF de L, Silva ÁEM da, Moreira SS, Assis GJ de F, Detmann E, Valadares Filho S de C, Fonseca Paulino M, Santos GM dos (2022) Effect of pre- and post-weaning supplementation on performance, nutritional, and metabolic characteristics in Nellore heifers under grazing (A Bach, Ed.). Animal Production Science 62, 1706–1719. doi:10.1071/AN22025.

Pérez-Torres L, Orihuela A, Corro M, Rubio I, Alonso MA, Galina CS (2016) Effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves. Applied Animal Behaviour Science 179, 17–22. doi:10.1016/j.applanim.2016.03.010.

Pérez-Torres L, Ortiz P, Martínez JF, Orihuela A, Rubio I, Corro M, Galina CS, Ungerfeld R (2021) Short- and long-term effects of temporary early cow–calf separation or restricted suckling on well-being and performance in zebu cattle. Animal 15, 100132. doi:10.1016/j.animal.2020.100132.

da Silva AG, Paulino MF, da Silva Amorim L, Rennó LN, Detmann E, de Moura FH, Manso MR, Silva e Paiva PH, Ortega REM, de Melo LP (2017) Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre- and post-weaning. Tropical Animal Health and Production 49, 707–715. doi:10.1007/s11250-017-1248-1.

Stěhulová I, Valníčková B, Šárová R, Špinka M (2017) Weaning reactions in beef cattle are adaptively adjusted to the state of the cow and the calf. Journal of Animal Science 95, 1023. doi:10.2527/jas2016.1207.

Ungerfeld R, Hötzel MJ, Quintans G (2015) Changes in behaviour, milk production and bodyweight in beef cows subjected to two-step or abrupt weaning. Animal Production Science 55, 1281. doi:10.1071/AN13453.

Ungerfeld R, Hötzel MJ, Scarsi A, Quintans G (2011) Behavioral and physiological changes in early-weaned multiparous and primiparous beef cows. Animal 5, 1270–1275. doi:10.1017/S1751731111000334.

Ungerfeld R, Quintans G, Enríquez DH, Hrötzel MJ (2009) Behavioural changes at weaning in 6-month-old beef calves reared by cows of high or low milk yield. Animal Production Science 49, 637. doi:10.1071/AN09037.

Vaz RZ, Restle J, Pacheco PS, Vaz FN, Pascoal LL, Vaz MB (2012) Ganho de peso pré e pós-desmame no desempenho reprodutivo de novilhas de corte aos quatorze meses de idade. Ciência Animal Brasileira 13, 272–281. doi:10.5216/cab.v13i3.17527.

**CHAPTER 1 - Does Parity Influence the Magnitude of the Stress Response of Nellore Cows at Weaning?** - Article published at the Animals (2023) - doi.org/10.3390/ani13081321

**Camila de Paula [1,] *, Luciana Navajas Rennó [1], Matheus Fellipe de Lana Ferreira [2], Samira Silveira Moreira [1], Hudson Caio Martins [1], Isabela Iria Rodrigues [1], Edenio Detmann [1], Sebastião de Campos Valadares Filho [1] and Mário Fonseca Paulino [1]**

## ABSTRACT

Most studies investigate the impact of stress at weaning on calves; however, little is known about the responses of cows, and whether they would differ according to parity. This study aims to investigate whether parity would influence the weaning stress response in beef cows. Thirty pregnant Nellore cows with their respective calves were randomly allocated to five paddocks and two females from each parity group were placed in the paddocks. There was an interaction ($p < 0.05$) between parity and evaluation days regarding cortisol, where on d +7, the higher concentration was observed for multiparous cows. There was an interaction ($p < 0.05$) between parity and evaluation day for red blood cells (RBC), hematocrit (HCT), and hemoglobin (HB), whereby higher RBC counts on d +4 were observed for multiparous cows. For HCT and HB, on all post-weaning collection days, higher values were observed for multiparous cows. The day of evaluation had an ($p < 0.05$) effect on all recorded behaviors, except for rumination ($p > 0.05$). Nellore cows, regardless of parity, underwent behavioral and physiological changes on abrupt weaning. Physiological parameters indicated that the magnitude of stress was greater in multiparous cows.

**Keywords:** cattle; behaviors; physiology; cortisol; number of vocalizations; ceruloplasmin; grazing; red blood cells

## INTRODUCTION

Weaning is a necessary and indispensable management process that allows the shortening of the production cycle as it permits the separation of the calf–cow pair earlier than would occur naturally. However, weaning is an extremely sensitive period from the production point of view as it is known to cause multiple stressors in the cow–calf pair [1–3].

Weaning triggers several physiological responses in calves, such as increased serum concentrations of cortisol and acute phase proteins. These physiological changes, which are negatively associated with the performance and health of calves, have been the focus of the majority of studies in this field [4–6]. However, the weaning period can also have an impact on the behavior of cows. For example, cows have been shown to decrease grazing time by up to 50% and increase vocalization, pacing, and time spent looking for offspring after weaning [7–10]. Nonetheless, few investigations of physiological responses have been carried out in cows [11,12].

There are many factors that have been identified as influencing the stress response to abrupt weaning in beef cows, such as the individual characteristics of the mother and offspring, including the age and weight gain of the calf, breed, parity, physiological state of the cow, and milk production [3,9,12]. For early weaning management, parity has been shown to influence the behavioral stress response in beef cows, with multiparous cows experiencing marked distress when separated from their calves; however, the multiparous cows were all in their second calving (secundiparous cows), so the authors could not con-firm whether, in cows with longer maternal experience (≥3 calvings), the differences would be similar or greater [12]. Multiparous cows exhibit better maternal behavior, as indicated by the fact that they nurse their calves more frequently [13] and produce more milk [14] than primiparous cows. The effects of parity on stress response at weaning seem to be related to the cow–calf bond, which is influenced by natural factors such as maternal ability and milk production. Therefore, the stronger the cow–calf bond, the more disruptive will be the weaning process.

Nevertheless, there is limited research that has examined weaning stress in the cow, and little is known about the extent of the effects of these physiological and behavioral changes. Further investigation is required to understand whether such responses are affected to the same extent when weaning is performed in the conventional way (at 7–8 months of age) and whether they are also influenced by parity. It is known that weaning stress affects the performance of beef cattle [15], and the metabolic status of the cow is influenced by parity, in which primiparous cows are more impacted by the lactation period, presenting more unbalanced metabolic and hormonal characteristics and lower body condition scores [16]. Therefore, understanding whether the response to stress in this period will have a negative impact on the productive life of the cow and whether it differs be-tween categories is essential to adopting appropriate management practices aiming at greater animal welfare and productive and reproductive performance.

Since no study has evaluated whether physiological and behavioral responses differ between primiparous, secundiparous, and multiparous cows, this study aimed to investigate the effect of parity on the weaning stress response in Nellore cows under grazing. Thus, we hypothesize that older cows will exhibit greater physiological and behavioral changes than young cows.

## MATERIALS AND METHODS

*Experimental Design and Animal Ethics*

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 No. 071/2019). The experiment was conducted at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20°45′S 42°52′W), between June and July 2019. The experimental period of 34 d was divided into a pre-weaning phase (20 d) and a post-weaning phase (14 d). The average rainfall during the data collection period was 0.0000 mm, and the maximum and minimum average temperatures were 18 °C and 17 °C, respectively.

Thirty pregnant Nellore cows with their respective female Nellore calves were used in the study. The average ages of the 10 primiparous, 10 secundiparous, and 10 multiparous cows were 2 years, 3 years, and 4–6 years, respectively, while the average body weights (BW) were 451 ± 61 kg, 492 ± 43 kg, and 537 ± 43 kg, respectively. The cows used in this study calved between September and October 2018 and came from the same beef herd. Cow–calf pairs were randomly allocated to five paddocks covered with Uruchloa decumbens grass (8.7 ha/paddock), and two females from each parity group were placed in the paddocks (six cow–calf pairs/paddock) 28 days before the beginning of the experiment to acclimate to the environment and social interactions.

All animals had free access to water and a commercial mineral mix ($CaHPO_4$, 50.00%; NaCl, 47.775%; $ZnSO_4$, 1.4%; $Cu_2SO_4$, 0.70%; $CoSO_4$, 0.05%; $KIO_3$, 0.05%; and $MnSO_4$, 0.025%). During the pre-weaning phase, calves were group-fed (six calves/paddock) with 6 g/kg BW of an energy-protein supplement containing 300 g crude protein (CP)/kg dry matter (DM) in a creep-feeding system. The supplement was formulated to supply approximately 50% of the CP requirement of calves, based on the BR-Corte system [17]. The supplement was provided daily at 1100 h. The average DM availability of forage for the experimental period was 5.0 t/ha (dry season). The chemical composition of forage by hand plucking was 380.7 g MS/kg of natural matter, 58.6 g CP/kg DM, 689.3 g neutral detergent fiber corrected for ash and protein/kg DM, 230.6 g indigestible neutral detergent fiber/kg DM, and 924.2 g organic matter/kg DM.

Calves were abruptly weaned (d 0) at an average age of 7.5 months. They were then loaded onto a livestock trailer and transported for 1.6 km to a different experimental area located at a sufficient distance away to avoid auditive interaction with the dams. The cows were kept in the same paddocks throughout the experiment, maintaining the social relationships established before weaning.

*Sampling and Analysis*

The cows were weighed (unshrunk BW) at d −5 before cow–calf separation (pre-weaning baseline) and at d +4, d +7, and d +14 (post-weaning) at 08:00 h. Simultaneously, blood 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 cortisol, total proteins, and albumin. The BD Vacutainer® Plus tube with sodium heparin was used for the quantification of haptoglobin and ceruloplasmin. After collection, samples were centrifuged at 2200× g for 20 min. Serum and plasma were frozen immediately and kept at −20 °C until they underwent analysis. Blood smears were prepared from whole blood with EDTA (BD Vacutainer® EDTA, São Paulo, Brazil) for hematological analysis.

The behavior of individual cows was recorded on d −3, +1, +3, and +6. Cows were tagged, and large numbers were painted on both sides of the body before observations were performed. The behavior was recorded by trained personnel (one per paddock), who were approximately 50 m away from the cows, using binoculars, to avoid the influence of human interference on the normal behavior of the cows [18,19]. Data were recorded from 06:00 h to 18:00 h by 15 evaluators, where each was responsible for one paddock for 4 consecutive hours. The observed behavioral characteristics were nursing time (on d −3 only), grazing, ruminating, trough time, idle time (lying and standing), walking with the head held high (looking for the calf), pacing, and vocalization (Table 1).

The number of observations of each behavior was transformed into percentages, with the different behaviors evaluated through the daytime totaling 100% (grazing, ruminating, trough time, idle time, walking, and pacing); nursing time was added for pre-weaning assessment only. The frequency of vocalizations was recorded for each cow and expressed as the number of vocalizations per day (each call was recorded as one occurrence).

Serum cortisol concentrations were detected by the chemiluminescent method (Beckman Coulter®, 33600, Brea, CA, USA) using the Access® 2 analyzer system (Beckman Coulter®, Brea, CA, USA). Blood concentrations of total protein (colorimetric kinetic test, Bioclin®, K031) and albumin (bromecresol green method, Bioclin® K040, Belo Horizonte, Brazil) were quantified using an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China).

Plasma ceruloplasmin was quantified by the oxidase activity method using colorimetric procedures, as described by Demetriou et al. [21]. Plasma haptoglobin concentration was quantified in duplicate samples by estimation of differences in peroxidase activity caused by haptoglobin–hemoglobin complexing [22]; the results were expressed as optical density resulting from readings at 450 nm × 100 [23].

Red blood cell (RBC) and white blood cell (WBC) count and hemoglobin concentration (HB) were quantified using the Hematoclin 2.8 Vet automatic analyzer (Bioclin®, Belo Horizonte, Brazil), the differentiation of leukocyte cells (neutrophils and lymphocytes) was determined under a microscope, and neutrophil to lymphocyte (N:L) ratios were calculated. Hematocrit (HCT) was quantified in a microcentrifuge (MicroSpin microhematocrit centrifuge, Jaboticabal, Brazil).

*Statistical Analysis*

The experiment was analyzed using the model:

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

where $Y_{ijk}$ is the observation taken on animal *k*, pertaining to parity *j*, and managed in the paddock *i*; μ is the overall constant; $P_i$ is the random effect of paddock *i*; $O_j$ is the fixed effect of parity *j*; and $e_{(ij)k}$ is the random error, assumed to be NIID (*0, σ2 e*).

All response variables were interpreted as repeated measurements [24] according to the evaluation days around the weaning time (fixed effect). The best structure of the residual (co)variance matrix was chosen based on Akaike's information criterion with correction. Degrees of freedom were estimated using the Kenward–Roger method. The analysis were performed using PROC MIXED in SAS 9.4 (Inst. Inc., Cary, NC, USA), with significance set at $p < 0.05$.

After a first round of analysis of variance, the experimental error was estimated and evaluated with regard to normal distribution and homoscedasticity (across parity and evaluation day) using the approaches provided by the Shapiro–Wilk and Levene tests, respectively. When these criteria were matched, pairwise comparisons were performed using the protected Fisher's least significant difference test.

We anticipated that experimental errors for vocalization would present neither normal distribution nor homogeneous variance ($p < 0.05$). Attempts were made to use the basic experimental model with different probability distributions for the errors through the GLIMMIX procedure of SAS 9.4. However, adequate convergence was not achieved. Hence, the pattern of vocalizations across evaluation days and parity was evaluated using a non-parametric Kruskal–Wallis (KW) approach via the NPAR1WAY procedure in SAS 9.4. When an overall significance for the KW test was achieved ($p < 0.05$), the Wilcoxon scores test was applied as a pairwise comparison procedure.

## RESULTS

### *Body Weight*

Analysis of the cows' BW showed that there was no interaction ($p = 0.343$) between parity and evaluation day (Figure 1). On average, multiparous cows presented a higher BW ($p < 0.0001$; SEM [Standard error of means] = 17.45), followed by secundiparous and primiparous cows. The BW of the cows changed according to the day of evaluation ($p < 0.0001$), with the lowest average BW observed just after the weaning.

### *Physiological Measurements*

Analysis of serum cortisol concentrations in the blood revealed an interaction ($p = 0.038$; SEM = 1.03) between parity and evaluation day (Table 2). Furthermore, differences between parity orders were detected (Figure 2a) on d +7, with higher cortisol concentrations detected in multiparous cows than in the other parity orders, which were similar to each other ($p > 0.05$). However, effects of day, but not parity or parity and day, were detected for ceruloplasmin ($p < 0.0001$; SEM = 0.99; Table 2) and haptoglobin concentrations ($p < 0.0001$;

SEM = 0.58; Table 2). In addition, ceruloplasmin (Figure 2b) and haptoglobin (Figure 2c) concentrations increased post-weaning and did not return to pre-weaning values on d +14.

Total protein ($p = 0.0003$; SEM = 0.17) and albumin ($p = 0.049$; SEM = 0.08) concentrations were affected by parity, with different values between the parity orders already in the pre-weaning phase (Table 2). Additionally, total protein ($p = 0.0002$) and albumin ($p = 0.0001$) concentrations were affected by day. Total protein concentrations were higher for multiparous and secundiparous cows than for primiparous cows, with the lowest concentrations detected on d +14 (Figure 3a). Furthermore, albumin levels were higher for multiparous cows than for primiparous cows and decreased from d +7, with lower concentrations on d +14 (Figure 3b).

For RBC count, there was an interaction ($p = 0.014$; 0.27) between parity and evaluation day (Table 2). Differences between parity orders were detected on d +4, whereby high-er RBC counts were observed for multiparous cows (Figure 4a) compared with secundiparous and primiparous cows, which did not differ from each other. An interaction was al-so observed (Table 2) between parity and evaluation days for HCT percentage ($p = 0.009$; SEM = 0.75). Differences between parity orders were detected on all post-weaning collection days (Figure 4b), with higher values being observed for multiparous cows than for primiparous cows. In addition, on d +4 and d +14, HCT values were higher for multiparous cows than for secundiparous cows. However, HCT values were only higher for secundiparous cows compared to primiparous cows on d +7. Analysis of HB concentration also revealed an interaction ($p = 0.034$; SEM = 0.29; Table 2) between parity and evaluation day. On all post-weaning collection days, HB concentration (Figure 4c) was higher for multiparous cows than for primiparous cows.

In addition, effects of day relative to weaning were detected for WBC counts and lymphocyte and neutrophil numbers (Table 2). All cows had lower WBC counts after weaning ($p < 0.0001$; SEM = 0.76; Figure 5a). Lymphocyte numbers decreased on d +4 and d +7 and increased again on d +14 ($p = 0.004$; SEM = 0.54; Figure 5b). Neutrophil numbers were also lower on d +4 ($p = 0.006$; SEM = 0.23; Figure 5c). Nonetheless, the N:L ratio was not affected by parity, day, or parity and day ($p > 0.05$; Table 2).

*Behavior Characteristics*

There were no differences in time spent nursing before weaning ($p > 0.05$; Table 3). In addition, behavioral observations indicated that there was no effect of parity or interaction between parity and evaluation day ($p > 0.05$; Table 3). However, the day of evaluation had a significant ($p < 0.05$) effect on all recorded behaviors, except for rumination ($p = 0.625$; SEM = 1.42). Time spent grazing decreased on d +1 after weaning, increasing on d +3 and d +6 but remaining lower than the pre-weaning values ($p < 0.0001$; SEM = 2.85; Figure 6a). Idle time increased after weaning ($p = 0.013$; SEM = 1.97; Figure 6b), while cows displayed lower trough time on the first day after weaning, returning to pre-weaning time on d +6 ($p = 0.037$; SEM0.35; Figure 6c). Pacing ($p < 0.0001$; SEM = 1.55; Figure 6d) and walking ($p < 0.0001$; SEM 0.59; Figure 6e) were higher on d +1 after weaning than on the other days.

Evaluation day also had an effect on the number of vocalizations by the cows (Table 3). On average, the cows vocalized more on the first day after separation from the calf ($p < 0.0001$; Figure 7a). As this specific day was the only one to present a significant number of vocalizations, we performed a nested evaluation to understand the differences between parity orders within the first day after weaning. However, despite a numerical trend whereby the older cows produced a greater number of vocalizations, no significant differences between parity orders were detected ($p = 0.580$; SEM = 17.86; Figure 7b).

## DISCUSSION

Weaning is a dynamic process, the underlying effects of which can impact different pathways. As such, assessing only changes in physiology, behavior, or performance pro-vides limited information [25]. Therefore, this study thoroughly evaluated weaning stress responses in beef cows using multiple approaches, enabling a broader view of the effects of weaning.

We showed that breaking the cow–offspring bond under conventional weaning management (weaning at 7 to 8 months) represented a stressful experience for Nellore cows, causing physiological and behavioral changes. Those changes were mostly observed during the early post-weaning period. Similarly, other authors have found behavioral responses to the stress of abrupt weaning to be short-lived, showing that most behaviors returned or were returning to pre-weaning frequencies by the third day after weaning [8,12]. Indeed, Lenner et al. [26] demonstrated that when cow and calf were brought together in the first week after weaning, the bond was stronger than when they were brought together during the third week.

Furthermore, in our study, although the behavior did seem not to be influenced by parity, there was physiological evidence that the magnitude of stress was greater for multiparous cows. Stěhulová et al. [9] showed that parity had no effect on the behavior of pregnant beef cows when weaning was performed at 7 months of age. The authors pointed out that reactions were reduced in pregnant cows or cows with older calves. As pregnant cows redirect resources to future offspring [27,28], the magnitude of behavioral changes is masked by these effects. Nonetheless, one of the first studies to evaluate stress responses to early weaning in beef cows of different parity orders showed that stress responses were stronger in multiparous cows, as indicated by greater changes in behavior such as walking and vocalizing. The authors concluded that weaning affected the welfare of multiparous cows more than that of primiparous cows, but these outcomes did not extend to physiological parameters [12]. It is noteworthy that in the experiment by Ungerfeld et al. [12], assessments were performed on multiparous cows that were all on their second calving, which corresponds to our secundiparous cows, and it is important to emphasize that secundiparous cows present intermediate characteristics between primiparous and multiparous. The differences between studies evaluating behavioral and physiological changes during abrupt weaning could be related to the different weaning ages, as in early weaning, the stress is more remarkable because of the stronger cow–calf bond [29]. In addition, they may be related to breeding, since cow temperament appears to influence the maternal ability [30] and, consequently, the stress of separation [3,31].

Differences in BW found between parity orders were expected because of age differences [32]. However, all categories showed reduced BW after weaning, which is indicative of stress caused by weaning. As the cows showed reduced trough and grazing time, the weight changes may have been related to lower water and feed intake, and thus less filling of the gastrointestinal tract [33]. In addition, the cows increased the amount of idle time, as well as time spent walking with their heads raised and time looking for their calves parallel to the fence. Cows have been reported to walk more when permanently separated from their calves [2] and to remain close to fences after weaning [8]. Thus, cows appear to spend more time looking for their calves than grazing, which may be intensified depending on the magnitude of the weaning stress [34]. This nutrient deprivation may stimulate the mobilization of body fat reserves and activate the hypothalamic–pituitary–adrenal axis [35,36], triggering an acute phase response in cattle [37,38]. Water and feed deprivation can also interfere with the ruminal environment, causing microbial death [39] and resulting in the release of endotoxins that can induce an acute phase response [4]. Thus, reduced dry matter intake has been identified as a contributor to acute phase responses and reduced performance in beef cattle [33,40–42].

Cortisol is indicated as a modulator of stress adaptation. One of the physiological effects of cortisol during the stress response is the triggering of nutrient mobilization from the liver, fat, and muscle [43]. This catabolic effect increases the amount of circulating nutrients available for the animal to deal with the stressor and restore homeostasis. However, defense cells interpret this tissue mobilization as a deviation from homeostasis and initiate the production of cytokines, which are the main stimulators of acute phase protein pro-duction in the liver [44]. The main positive acute phase proteins that increase during inflammation or stress in cattle are haptoglobin, ceruloplasmin, and amyloid A [42,45–47]. Our results showed that ceruloplasmin and haptoglobin concentrations increased after weaning in all cows, regardless of parity, although increased cortisol was only detected in multiparous cows. Similarly, Lynch et al. [11] showed that haptoglobin concentration was increased in primiparous cows after weaning, without changes in cortisol concentration.

Differences found in total proteins and albumin concentrations according to parity are not related to weaning stress. According to Ferreira et al. [16], parity directly influences all indicators of protein status (i.e., total proteins, albumin, globulins, urea, and IGF-1) in beef cows. Thus, protein concentrations are expected to be lower for primiparous cows, as this category requires nutrients for fetal development, lactation, and continued growth. Nonetheless, regardless of category, there are two possible reasons that may explain the reduction in albumin concentration on days +7 and +14 after weaning. As previously de-scribed, there was a reduction in grazing, implying that protein intake was decreased, which could have led to lower serum albumin concentrations [48]. At the same time, this reduction may have been potentiated by the stress of weaning, as albumin is also known as a negative acute phase protein [46]. Determination of acute phase protein levels is used to monitor the health and welfare of animals in large herds [47]. Thus, low albumin concentration and high levels of haptoglobin and ceruloplasmin can be indicative of distress in cows when separated from their calves.

The increases in RBC count, HB concentration, and HCT percentage for multiparous cows after weaning did not appear to be related to polycythemia. Instead, these altered parameters may have been a reflection of lower water intake, although trough time was similarly reduced in the different parity orders. Moreover, it should be noted that multiparous cows had a cortisol peak, suggesting that this increase serves to protect and maintain water balance in times of stress [49]. Similarly, Campistol et al. [6] reported increases in RBCs and HCT in calves after weaning. Additionally, alterations in WBC population components occurred regardless of parity, indicating an attempt to restore homeostasis. WBC numbers were reduced, with lower numbers of lymphocytes (the main WBC type in cattle), neutrophils, and monocytes (data not shown). According to Lynch et al. [11], in beef cows, leukocyte and lymphocyte numbers decrease after weaning, which is characteristic of a stress leukogram. However, despite the post-weaning reductions, the RBC and WBC counts reported for all cows were in line with the reference limits for beef cows [50].

Previous studies from our group found that parity influenced milk production and composition in beef cows. Milk yields were higher in multiparous animals than in primiparous and secundiparous cows [14], in line with other studies [51,52]. Maternal milk output represents the costliest investment of the cow in the growth of her calf. Thus, mothers of calves with greater weight gain would experience greater marked suffering because milk is the most important factor in the weight gain of their calves. Grazing time has also been observed to be higher for calves born to primiparous and secundiparous cows than for those born to older animals [53]. Calves born to cows with lower milk production tend to increase grazing in an attempt to consume similar amounts of metabolizable energy per unit weight [54,55]. Conversely, calves of multiparous cows are expected to receive more milk as a result of the higher milk production of the dams; therefore, the cow–calf bond should be greater in such pairs. It is interesting to note that our findings were consistent with these results, indicating that female calves born to one- or two-calf cows exhibit more independence and thus, weaker cow–calf bonds. This may have contributed to the lower stress responses in these cows during weaning. Nonetheless, a previous study indicated that weight gain in calves was less important than the age of the calf at weaning in terms of effects on the behavior of cows [9].

In summary, our study is the first to show that in Nellore cows, behavioral stress responses to abrupt weaning were independent of previous maternal experiences and were of short duration. They were characterized mainly by the initiation of pacing, walking, and vocalization, and reduced grazing and trough time on the first day after cow–calf separation. In contrast, physiological changes lasted longer and were more pronounced in multiparous cows who displayed higher cortisol levels, erythrocyte numbers, HB concentrations, and HCT percentages. With these findings, specific practices that aim to improve the well-being and reproductive performance of cows, especially multiparous cows, can be developed and adapted to avoid the mistaken use of management that was generated from information on other breeds, other types of weaning, and environments. More research on this topic should be developed to establish specific practical recommendations for these animals, such as the use of alternative methods to abrupt weaning for multiparous cows.

**CONCLUSIONS**

Nellore cows, regardless of parity, underwent behavioral and physiological changes as a result of abrupt weaning. Additionally, physiological parameters indicated that the magnitude of stress was greater in multiparous cows.

**REFERENCES**


1. Weary, D.M.; Jasper, J.; Hötzel, M.J. Understanding Weaning Distress. Applied Animal Behaviour Science 2008, 110, 24–41. https://doi.org/10.1016/j.applanim.2007.03.025.
2. Ungerfeld, R.; Hötzel, M.J.; Quintans, G. Changes in Behaviour, Milk Production and Bodyweight in Beef Cows Subjected to Two-Step or Abrupt Weaning. Anim. Prod. Sci. 2015, 55, 1281. https://doi.org/10.1071/AN13453.
3. Lynch, E.; McGee, M.; Earley, B. Weaning Management of Beef Calves with Implications for Animal Health and Welfare. Journal of Applied Animal Research 2019, 47, 167–175. https://doi.org/10.1080/09712119.2019.1594825.
4. Carroll, J.A.; Arthington, J.D.; Chase, C.C. Early Weaning Alters the Acute-Phase Reaction to an Endotoxin Challenge in Beef Calves1. Journal of Animal Science 2009, 87, 4167–4172. https://doi.org/10.2527/jas.2009-2016.
5. Lynch, E.M.; McGee, M.; Doyle, S.; Earley, B. Effect of Post-Weaning Management Practices on Physiological and Immunological Responses of Weaned Beef Calves. Irish Journal of Agricultural and Food Research 2011, 50.
6. Campistol, C.; Kattesh, H.G.; Waller, J.C.; Rawls, E.L.; Arthington, J.D.; Carroll, J.A.; Pighetti, G.M.; Saxton, A.M. Effects of Pre-Weaning Feed Supplementation and Total versus Fenceline Weaning on the Physiology and Performance of Beef Steers. Int. J. Livest. Prod. 2016, 7, 48–54. https://doi.org/10.5897/IJLP2016.0291.
7. Ungerfeld, R.; Quintans, G.; Hötzel, M.J. Minimizing Cows' Stress When Calves Were Early Weaned Using the Two-Step Method with Nose Flaps. Animal 2016, 10, 1871–1876. https://doi.org/10.1017/S1751731116000793.
8. Pérez-Torres, L.; Orihuela, A.; Corro, M.; Rubio, I.; Alonso, M.A.; Galina, C.S. Effects of Separation Time on Behavioral and Physiological Characteristics of Brahman Cows and Their Calves. Applied Animal Behaviour Science 2016, 179, 17–22. https://doi.org/10.1016/j.applanim.2016.03.010.
9. Stěhulová, I.; Valníčková, B.; Šárová, R.; Špinka, M. Weaning Reactions in Beef Cattle Are Adaptively Adjusted to the State of the Cow and the Calf. Journal of Animal Science 2017, 95, 1023. https://doi.org/10.2527/jas2016.1207.
10. Pérez-Torres, L.; Ortiz, P.; Martínez, J.F.; Orihuela, A.; Rubio, I.; Corro, M.; Galina, C.S.; Ungerfeld, R. Short- and Long-Term Effects of Temporary Early Cow–Calf Separation or Restricted Suckling on Well-Being and Performance in Zebu Cattle. animal 2021, 15, 100132. https://doi.org/10.1016/j.animal.2020.100132.

11. Lynch, E.M.; Earley, B.; McGee, M.; Doyle, S. Characterisation of Physiological and Immunological Responses in Beef Cows to Abrupt Weaning and Subsequent Housing. BMC Vet Res 2010, 6, 37. https://doi.org/10.1186/1746-6148-6-37.
12. Ungerfeld, R.; Hötzel, M.J.; Scarsi, A.; Quintans, G. Behavioral and Physiological Changes in Early-Weaned Multiparous and Primiparous Beef Cows. Animal 2011, 5, 1270–1275. https://doi.org/10.1017/S1751731111000334.
13. Stěhulová, I.; Špinka, M.; Šárová, R.; Máchová, L.; Kněz, R.; Firla, P. Maternal Behaviour in Beef Cows Is Individually Consistent and Sensitive to Cow Body Condition, Calf Sex and Weight. Applied Animal Behaviour Science 2013, 144, 89–97. https://doi.org/10.1016/j.applanim.2013.01.003.
14. Ferreira, M.F. de L.; Rennó, L.N.; Rodrigues, I.I.; Valadares Filho, S. de C.; Costa e Silva, L.F.; Silva, F.F. e; Detmann, E.; Paulino, M.F. Evaluation of Non-Linear Models to Predict Potential Milk Yield of Beef Cows According to Parity Order Under Grazing. Front. Vet. Sci. 2021, 8, 721792. https://doi.org/10.3389/fvets.2021.721792.
15. Orihuela, A.; Galina, C.S. Effects of Separation of Cows and Calves on Reproductive Performance and Animal Welfare in Tropical Beef Cattle. Animals 2019, 9, 223. https://doi.org/10.3390/ani9050223.
16. Ferreira, M.F. de L.; Rennó, L.N.; Rodrigues, I.I.; Detmann, E.; Paulino, M.F.; de Campos Valadares Filho, S.; Martins, H.C.; Moreira, S.S.; de Lana, D.S. Effects of Parity Order on Performance, Metabolic, and Hormonal Parameters of Grazing Beef Cows during Pre-Calving and Lactation Periods. BMC Vet Res 2021, 17, 311. https://doi.org/10.1186/s12917-021-03019-0.
17. Valadares Filho, S. de C.; Silva, L.F.C. e; Gionbelli, M.P.; Rotta, P.P.; Marcondes, M.I.; Chizzotti, M.L.; Prados, L.F. Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE; 3rd ed.; Editora Federal de Viçosa, 2016; ISBN 978-85-8179-111-1.
18. Martins, L.S.; Paulino, M.F.; Rennó, L.N.; Detmann, E.; de Almeida, D.M.; Ortega, R.M.; Moreno, D.P.S.; Cárdenas, J.E.G. Creep Feeding Effects on Male Nellore Calves Influencing Behavior and Performance of Their Dams. Trop Anim Health Prod 2017, 49, 1669–1676. https://doi.org/10.1007/s11250-017-1375-8.
19. Lopes, S.A.; Paulino, M.F.; Detmann, E.; Valente, É.E.L.; Rennó, L.N.; Valadares, R.F.D.; Cardenas, J.E.G.; Almeida, D.M. de; Moura, F.H. de; Oliveira, C.A.S. Evaluation of Supplementation Plans for Suckling Beef Calves Managed on Tropical Pasture. SCA 2017, 38, 1027. https://doi.org/10.5433/1679-0359.2017v38n2p1027.

20. Ungerfeld, R.; Quintans, G.; Enríquez, D.H.; Hrötzel, M.J. Behavioural Changes at Weaning in 6-Month-Old Beef Calves Reared by Cows of High or Low Milk Yield. Anim. Prod. Sci. 2009, 49, 637. https://doi.org/10.1071/AN09037.
21. Demetriou, J.A.; Drewes, P.A.; Gin, J.B. Ceruloplasmin. In Clinical Chemistry; Cannon, D.C., Winkelman, J.W.: Harper and Row, Hagerstown, MD, 1974; pp. 857–864.
22. Makimura, S.; Suzuki, N. Quantitative Determination of Bovine Serum Haptoglobin and It Elevation in Some Inflammatory Disease. Japanese Journal of Veterinary Science 1982, 44, 15–21.
23. Cooke, R.F.; Arthington, J.D. Concentrations of Haptoglobin in Bovine Plasma Determined by ELISA or a Colorimetric Method Based on Peroxidase Activity: Methods to Determine Haptoglobin in Bovine Plasma. Journal of Animal Physiology and Animal Nutrition 2013, 97, 531–536. https://doi.org/10.1111/j.1439-0396.2012.01298.x.
24. Kaps, M.; Lamberson, W.R. Biostatistics for Animal Science; CABI Pub: Wallingford, Oxfordshire; Cambridge, MA, 2004; ISBN 978-0-85199-820-6.
25. Bath, G.F. Management of Pain in Production Animals. Applied Animal Behaviour Science 1998, 59, 147–156. https://doi.org/10.1016/S0168-1591(98)00129-4.
26. Lenner, Á.; Ragán, P.; Komlósi, I. Study of Changes in the Strength of Connection between Grey Cattle Cows and Their Offspring after Weaning. Acta agrar. Debr. 2021, 129–136. https://doi.org/10.34101/actaagrar/1/8468.
27. Green, W.C.H.; Griswold, J.G.; Rothstein, A. Post-Weaning Associations among Bison Mothers and Daughters. Animal Behaviour 1989, 38, 847–858. https://doi.org/10.1016/S0003-3472(89)80116-2.
28. Bateson, P. The Dynamicsof Parent-Offspring Relationships in Mammals. 1994.
29. Enríquez, D.; Hötzel, M.J.; Ungerfeld, R. Minimising the Stress of Weaning of Beef Calves: A Review. Acta Vet Scand 2011, 53, 28. https://doi.org/10.1186/1751-0147-53-28.
30. Pires, B.V.; Freitas, L.A. de; Silva, G.V. da; Mendonça, G.G.; Savegnago, R.P.; Lima, M.L.P. de; Faro, L.E.; Cyrillo, J.N. dos S.G.; Paz, C.C.P. de Maternal-Offspring Behavior of Guzerat Beef Cattle. Pesq. agropec. bras. 2020, 55, e01504. https://doi.org/10.1590/s1678-3921.pab2020.v55.01504.

31. Burdick, N.C.; Randel, R.D.; Carroll, J.A.; Welsh, T.H. Interactions between Temperament, Stress, and Immune Function in Cattle. International Journal of Zoology 2011, 2011, 1–9. https://doi.org/10.1155/2011/373197.

32. Linden, D.R.; Titgemeyer, E.C.; Olson, K.C.; Anderson, D.E. Effects of Gestation and Lactation on Forage Intake, Digestion, and Passage Rates of Primiparous Beef Heifers and Multiparous Beef Cows1. Journal of Animal Science 2014, 92, 2141–2151. https://doi.org/10.2527/jas.2013-6813.

33. Marques, R.S.; Cooke, R.F.; Francisco, C.L.; Bohnert, D.W. Effects of Twenty-Four Hour Transport or Twenty-Four Hour Feed and Water Deprivation on Physiologic and Performance Responses of Feeder Cattle1. Journal of Animal Science 2012, 90, 5040–5046. https://doi.org/10.2527/jas.2012-5425.

34. Price, E.O.; Harris, J.E.; Borgwardt, R.E.; Sween, M.L.; Connor, J.M. Fenceline Contact of Beef Calves with Their Dams at Weaning Reduces the Negative Effects of Separation on Behavior and Growth Rate1. Journal of Animal Science 2003, 81, 116–121. https://doi.org/10.2527/2003.811116x.

35. Ward, J.R.; Henricks, D.M.; Jenkins, T.C.; Bridges, W.C. Serum Hormone and Metabolite Concentrations in Fasted Young Bulls and Steers. Domestic Animal Endocrinology 1992, 9, 97–103. https://doi.org/10.1016/0739-7240(92)90023-Q.

36. Cooke, R.F.; Del Río, N.S.; Caraviello, D.Z.; Bertics, S.J.; Ramos, M.H.; Grummer, R.R. Supplemental Choline for Prevention and Alleviation of Fatty Liver in Dairy Cattle. Journal of Dairy Science 2007, 90, 2413–2418. https://doi.org/10.3168/jds.2006-028.

37. Cooke, R.F.; Bohnert, D.W. Technical Note: Bovine Acute-Phase Response after Corticotrophin-Release Hormone Challenge1. Journal of Animal Science 2011, 89, 252–257. https://doi.org/10.2527/jas.2010-3131.

38. Cooke, R.F.; Carroll, J.A.; Dailey, J.; Cappellozza, B.I.; Bohnert, D.W. Bovine Acute-Phase Response after Different Doses of Corticotropin-Releasing Hormone Challenge1,2,3. Journal of Animal Science 2012, 90, 2337–2344. https://doi.org/10.2527/jas.2011-4608.

39. Meiske, J.C.; Salsbury, R.L.; Hoefer, J.A.; Luecke, R.W. The Effect of Starvation and Subsequent Refeeding on Some Activities of Rumen Microorganisms In Vitro. Journal of Animal Science 1958, 17, 774–781. https://doi.org/10.2527/jas1958.173774x.

40. Gruys, E.; Toussaint, M.J.M.; Niewold, T.A.; Koopmans, S.J. Acute Phase Reaction and Acute Phase Proteins. J Zheijang Univ Sci B 2005, 6, 1045–1056. https://doi.org/10.1631/jzus.2005.B1045.

41. Arthington, J.D.; Qiu, X.; Cooke, R.F.; Vendramini, J.M.B.; Araujo, D.B.; Chase, C.C.; Coleman, S.W. Effects of Pre shipping Management on Measures of Stress and Performance of Beef Steers during Feedlot Receiving1. Journal of Animal Science 2008, 86, 2016–2023. https://doi.org/10.2527/jas.2008-0968.

42. Cooke, R.F. Invited Paper: Nutritional and Management Considerations for Beef Cattle Experiencing Stress-Induced Inflammation 1 1This Article Was Based on a Presentation at the ARPAS Symposium "Understanding Inflammation and Inflammatory Biomarkers to Improve Animal Performance" at the 2016 Joint Annual Meeting, July 19–23, 2016, Salt Lake City, Utah. The Professional Animal Scientist 2017, 33, 1–11. https://doi.org/10.15232/pas.2016-01573.

43. Nelson, D.L.; Cox, M.M. Lehninger: Principles of Biochemistry; 4th ed.; WH Freeman and Company, New York, USA, 2005; ISBN 0-7167-4339-6.

44. Carroll, J.A.; Forsberg, N.E. Influence of Stress and Nutrition on Cattle Immunity. Veterinary Clinics of North America: Food Animal Practice 2007, 23, 105–149. https://doi.org/10.1016/j.cvfa.2007.01.003.

45. Godson, D.L.; Baca-Estrada, M.E.; Kessel, A.G.V.; Hughes, H.P.A.; Morsy, M.A.; Van, J.; Harland, R.J.; Shuster, D.E.; Daley, M.J.; Babiuk, L.A. Regulation of Bovine Acute Phase Responses by Recombinant Interleukin- 11, 1995.

46. Gomez-Laguna, J.; Salgueiro, F.J.; Pallarés, F.J.; Rodríguez-Gómez, I.M.; Barranco, I.; Carrasco, L. Acute Phase Proteins as Biomarkers in Animal Health and Welfare. In Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases; Veas, F., Ed.; InTech, 2011 ISBN 978-953-307-873-1.

47. Paulina, J.; Tadeusz, S. Acute Phase Proteins in Cattle. In Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases; Veas, F., Ed.; InTech, 2011 ISBN 978-953-307-873-1.

48. Payne, J.M.; Payne, S. The metabolic profile test; Oxford University Press, Oxford, UK, 1987; ISBN 978-0-19-854544-6.

49. Parker, A.J.; Hamlin, G.P.; Coleman, C.J.; Fitzpatrick, L.A. Excess Cortisol Interferes with a Principal Mechanism of Resistance to Dehydration in Bos Indicus Steers. 2004.

50. Jones, M.L.; Allison, R.W. Evaluation of the Ruminant Complete Blood Cell Count. Veterinary Clinics of North America: Food Animal Practice 2007, 23, 377–402. https://doi.org/10.1016/j.cvfa.2007.07.002.
51. Neville, W.E.; Warren, E.P.; Griffey, W.A. Estimates of Age Effects on Milk Production in Hereford Cows. Journal of Animal Science 1974, 38, 1–5. https://doi.org/10.2527/jas1974.3811.
52. Pimentel, M.A.; Moraes, J.C.F.; Jaume, C.M.; Lemes, J.S.; Brauner, C.C. Características da lactação de vacas Hereford criadas em um sistema de produção extensivo na região da campanha do Rio Grande do Sul. R. Bras. Zootec. 2006, 35, 159–168. https://doi.org/10.1590/S1516-35982006000100021.
53. Rodrigues, I.I. Comportamento Pré e Pós-Parto de Fêmeas Nelore de Diferentes Ordens de Parto e Suas Bezerras Em Pastejo. Dissertation in Animal Science, Universidade Federal de Viçosa: Minas Gerais, Brazil, 2020.
54. Baker, R.D.; Le Du, Y.L.P.; Barker, J.M. Milk-Fed Calves: 1. The Effect of Milk Intake upon the Herbage Intake and Performance of Grazing Calves. J. Agric. Sci. 1976, 87, 187–196. https://doi.org/10.1017/S0021859600026745.
55. Ansotegui, R.P.; Havstad, K.M.; Wallace, J.D.; Hallford, D.M. Effects of Milk Intake on Forage Intake and Performance of Suckling Range Calves. Journal of Animal Science 1991, 69, 899. https://doi.org/10.2527/1991.693899x.

## TABLES AND FIGURES

**Table 1.** Description of observed cattle behaviors in pasture.

| **Behavior** | **Definition** * |
|---|---|
| Grazing (% [1]) | Picking or consuming pasture, with the head above ground |
| Idle (%[1]) | In any resting position |
| Ruminating (% [1]) | Chewing regurgitated boluses of feed |
| Trough (% [1]) | Going to the trough in search of food or water |
| Pacing (% [1]) | Moves looking for the calf parallel to the fence, at 1 m |
| Walking (% [1]) | All four legs are moved with the head raised |
| Nursing (% [1]) | The calf suckles the udder |
| Vocalization [2] | Emission of characteristic sounds through the mouth |

* Adapted from [20]. [1] % = percentage of observation time; [2] Number of vocalizations.

**Table 2.** Effect of parity on physiological measurements in Nellore cows at abrupt weaning.

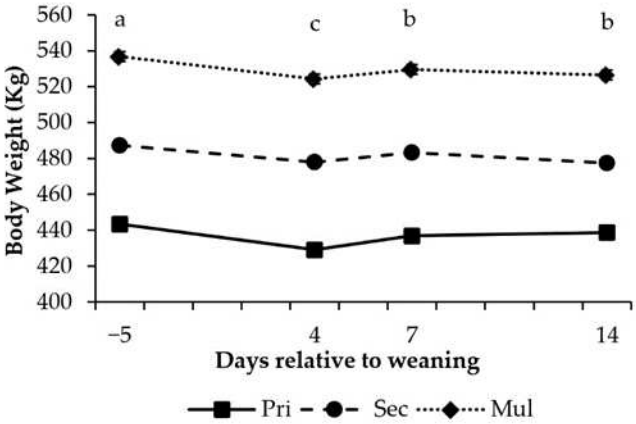
| Item | Parity | | | SEM | *p*-Value | | |
|---|---|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Par | Day [1] | Par × Day |
| Cortisol, ng/mL | 7.33 | 6.63 | 9.00 | 1.031 | 0.198 | 0.174 | 0.038 |
| Ceruloplasmin, mg/dL | 12.60 | 11.52 | 10.36 | 0.986 | 0.157 | <0.0001 | 0.372 |
| Haptoglobin, DO×100 | 3.33 | 3.44 | 3.23 | 0.579 | 0.578 | <0.0001 | 0.081 |
| Total proteins, g/dL | 6.79 [b] | 7.29 [a] | 7.45 [a] | 0.165 | 0.0003 | 0.0002 | 0.196 |
| Albumin, g/dL | 2.94 [b] | 3.09 [ab] | 3.10 [a] | 0.079 | 0.049 | 0.0001 | 0.668 |
| RBC, $\times 10^6/\mu L$ | 7.55 | 7.57 | 7.81 | 0.207 | 0.337 | 0.287 | 0.014 |
| HCT, % | 33.05 | 34.94 | 36.71 | 0.748 | 0.001 | 0.011 | 0.009 |
| HB, g/dL | 10.33 | 11.02 | 11.55 | 0.292 | 0.001 | 0.002 | 0.034 |
| WBC, $\times 10^3/\mu L$ | 12.25 | 11.79 | 11.86 | 0.756 | 0.866 | <0.0001 | 0.390 |
| Lymphocytes, $\times 10^3/\mu L$ | 7.07 | 7.03 | 6.92 | 0.538 | 0.974 | 0.004 | 0.947 |
| Neutrophils, $\times 10^3/\mu L$ | 3.53 | 3.32 | 3.39 | 0.234 | 0.777 | 0.006 | 0.375 |
| N:L | 0.51 | 0.48 | 0.53 | 0.033 | 0.542 | 0.201 | 0.947 |

OD = Optical Density; RBC = Red Blood Cell number; HCT = Hematocrit percentage; HB = Hemoglobin concentration; WBC = White Blood Cell number; N: L = Neutrophil/Lymphocyte relationship; SEM = Standard error of means; Par = Parity. [1] Day relative to weaning. a–b Different letters declare significantly different between parities at $p < 0.05$.

**Table 3.** Effect of parity on behavior observation in Nellore cows at abrupt weaning.

| Item | Parity | | | SEM | *p*-Value | | |
|---|---|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Par | Day [1] | Par × Day |
| Grazing, % | 55.71 | 53.41 | 52.62 | 2.853 | 0.515 | <0.0001 | 0.613 |
| Idle, % | 17.95 | 20.41 | 21.82 | 1.973 | 0.259 | 0.013 | 0.959 |
| Ruminating, % | 20.34 | 19.34 | 19.17 | 1.418 | 0.769 | 0.625 | 0.411 |
| Trough, % | 1.47 | 1.64 | 1.75 | 0.348 | 0.729 | 0.037 | 0.926 |
| Pacing, % | 2.97 | 3.21 | 2.22 | 1.551 | 0.847 | <0.0001 | 0.995 |
| Walking, % | 1.10 | 1.78 | 1.64 | 0.587 | 0.091 | <0.0001 | 0.798 |
| Nursing [2], % | 1.58 | 1.58 | 1.72 | 0.503 | 0.962 | - | - |
| Number of vocalizations | 15.27 | 20.5 | 33.18 | 17.860 | 0.580 | | |

SEM = Standard error of means; Par = Parity. [1] Day relative to weaning. [2] nursing time was added for pre-weaning assessment only.




Parity P<.0001
Day P<.0001
ParityxDay P=0.343

**Figure 1.** The body weight of Nellore cows with different parity under grazing according to the days relative to weaning. Days with different superscripts differ from each other for all parity orders ($p < 0.05$).

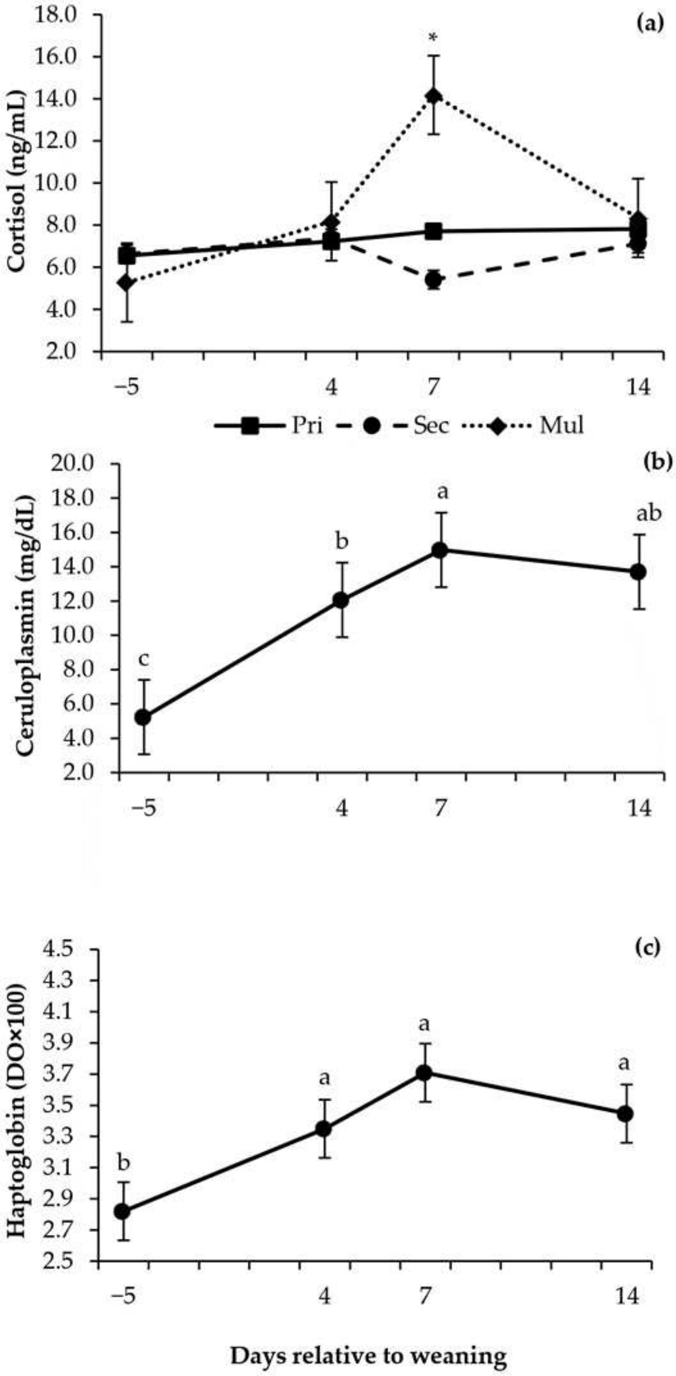



**Figure 2.** Cortisol (a), ceruloplasmin (b), and haptoglobin (c) serum concentrations in Nellore cows with different parity under grazing according to the days relative to weaning. Days with different superscripts differ from each other for all parity orders ($p < 0.05$). On days with asterisks (*), there is a parity × day interaction ($p < 0.05$).

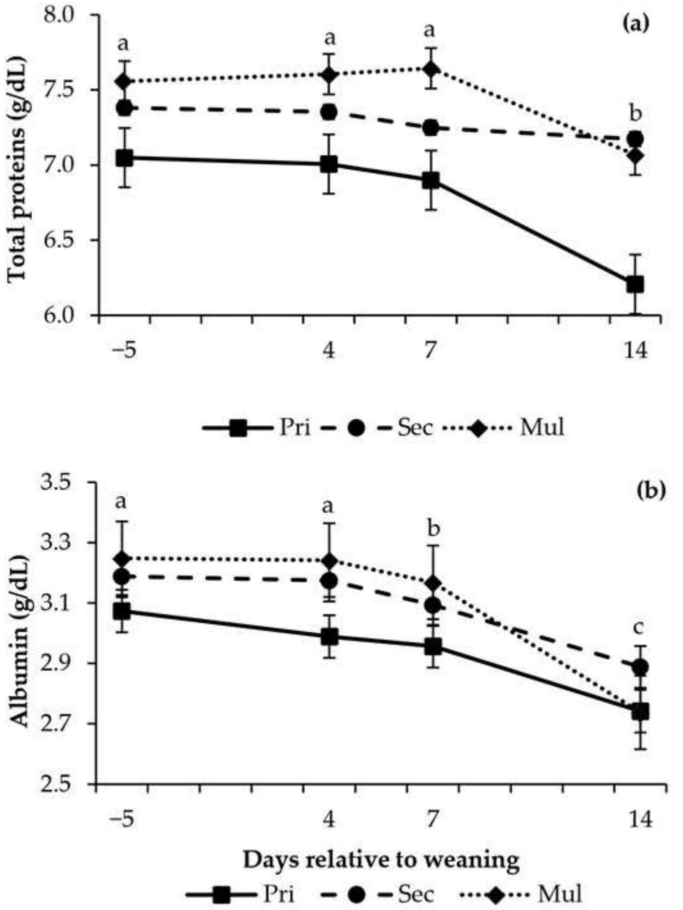



**Figure 3.** Total proteins (a) and albumin (b) serum concentrations in Nellore cows with different parity under grazing according to the days relative to weaning. Days with different superscripts differ from each other for all parity orders ($p < 0.05$).

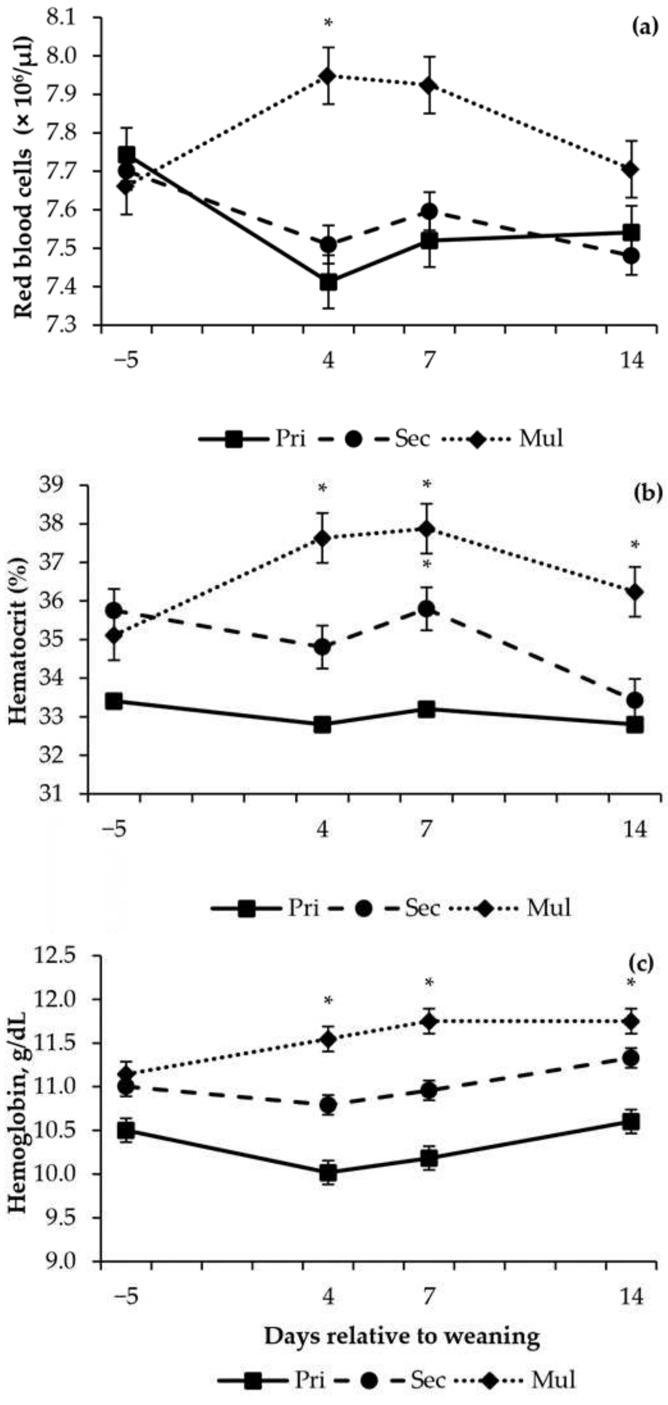



**Figure 4.** Red blood cell number (RBC; (a)), Hematocrit percentage (HCT; (b)), and hemoglobin concentration (HB; (c)) in Nellore cows with different parity under grazing according to the days relative to weaning. On days with asterisks (*) there is a parity x day interaction ($p < 0.05$).

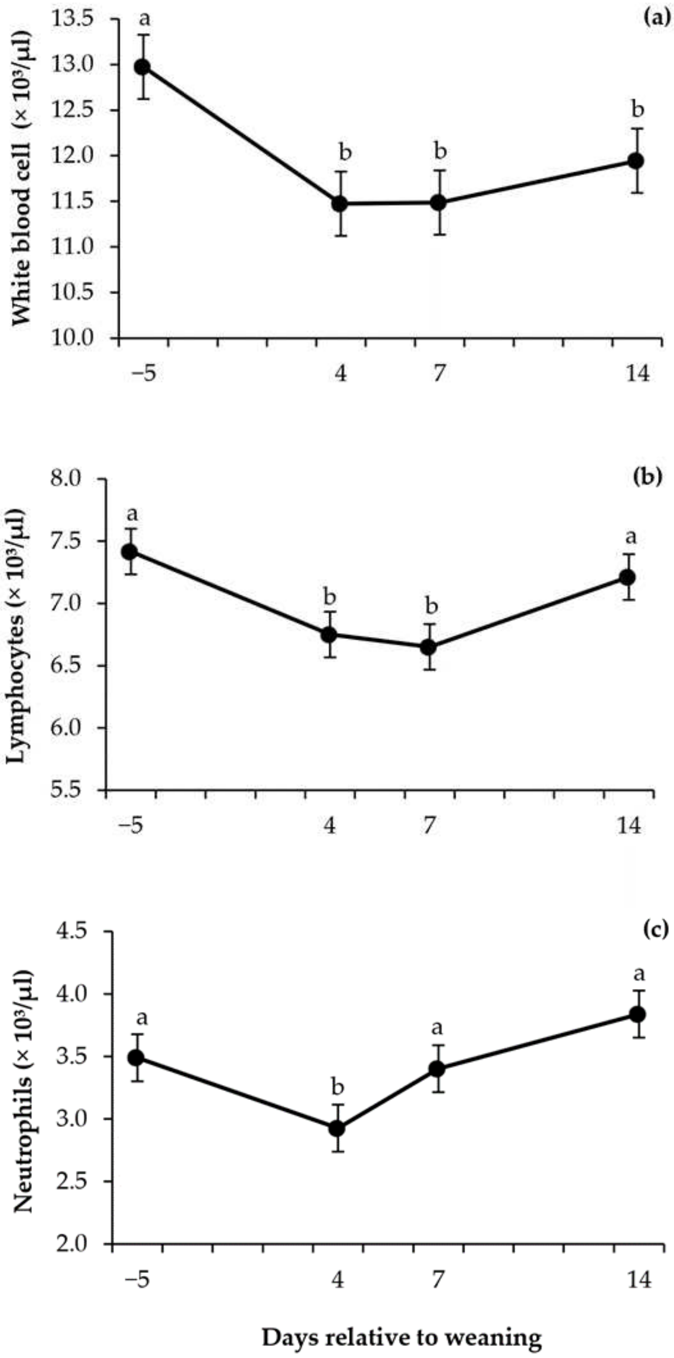



**Figure 5.** White blood cell number (WBC; (a)), lymphocyte number (b), and neutrophil number (c) in Nellore cows with different parity under grazing according to the days relative to weaning. Days with different superscripts differ from each other for all parity orders ($p < 0.05$).

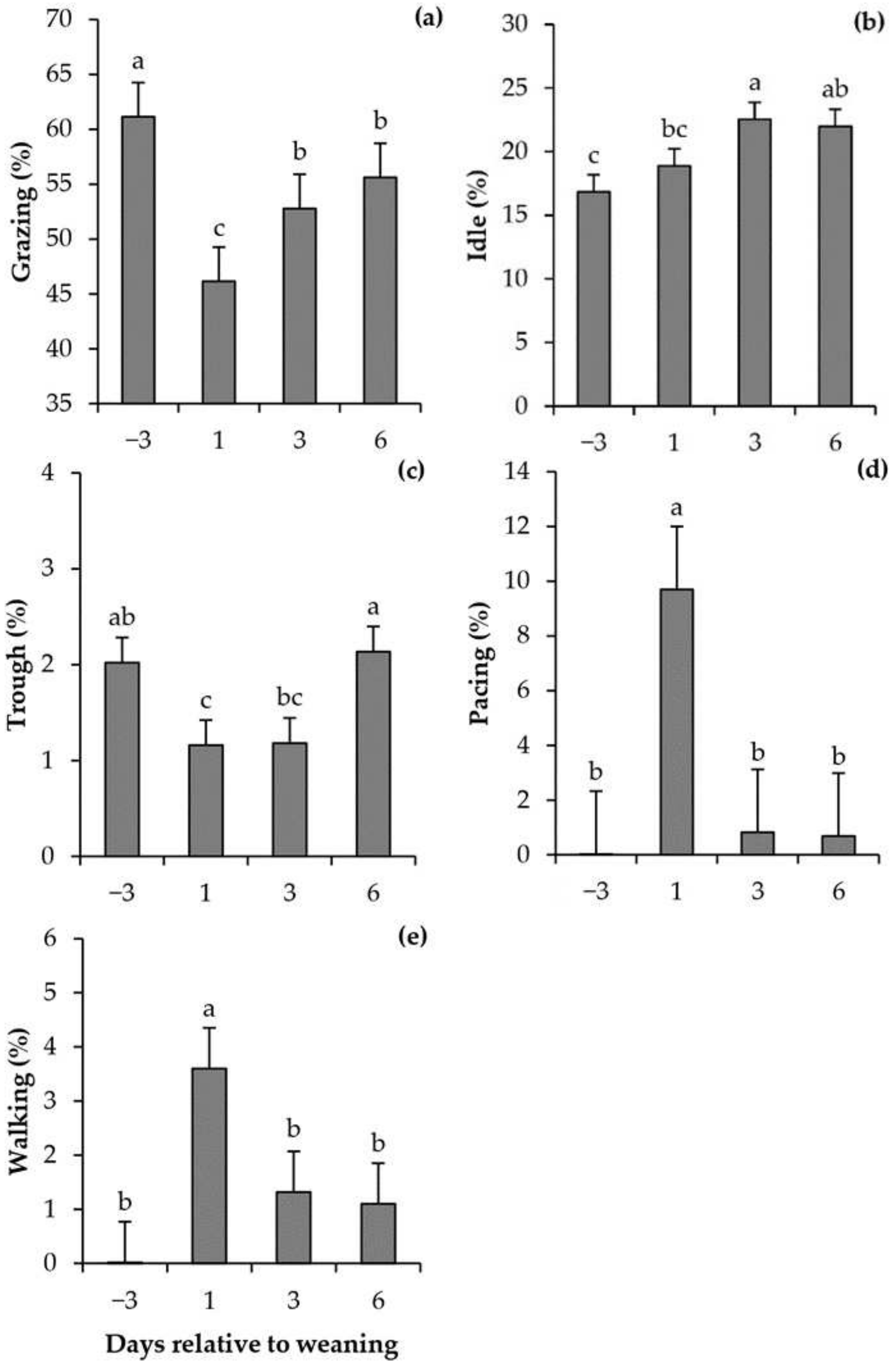



**Figure 6.** Grazing (a), idle time (b), trough time (c), pacing (d), and walking (e) in Nellore cows under grazing according to the days relative to weaning. Days with different superscripts differ from each other ($p < 0.05$).

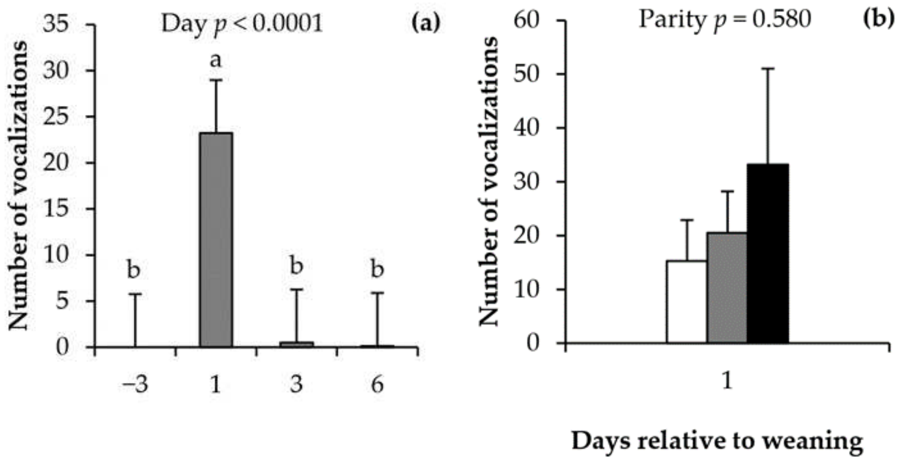



**Figure 7.** The number of vocalizations recorded in Nellore cows under grazing according to the days relative to weaning (a) and in primiparous (white), secundiparous (grey), and multiparous cows (black) on d +1 after weaning (b). Days with different superscripts differ from each other ($p < 0.05$).

**CHAPTER 2 - Does parity of the dams influence the magnitude of the stress response of Nellore heifers at abrupt weaning? -** In preparation to Animal Bioscience.

**ABSTRACT**

**Objective:** There is a lack of information about the physiological changes that occur at weaning in Nellore female calves, and whether they differ according to the parity of the dams. Accordingly, this study aimed to evaluate the influence of the dam's parity on the magnitude of stress response to abrupt weaning of Nellore heifers under grazing.
**Methods:** Thirty Nellore female calves with their respective dams were divided according to the parity order of the dams: 10 primiparous (2 years), 10 secundiparous (3 years), and 10 multiparous (4–6 years). Cow-calf pairs were randomly allocated to five paddocks, where two heifers from each group were placed in the paddocks. Female calves were abruptly weaned (d 0) at an average age of 225 ± 7 days. The heifers were weighed at d -5 (pre-weaning baseline), d 0 (5 hours after weaning), d +1, d +3, d +7, and d +14 (post-weaning), and simultaneously, blood samples were collected. All the statistical evaluations were performed considering 0.05 as the critical level of probability for the occurrence of the type I error.
**Results:** In the pre-weaning phase, the initial body weight (BW), final BW, and average daily gain of heifers were similar between parities ($p>0.05$). In response to weaning stress, BW was not affected by the interaction ($p>0.05$), however, it was affected by day ($p<0.05$). Only the cortisol concentration of the heifers was influenced by the dam's parity ($p<0.05$), with a higher concentration for heifers born to multiparous than primiparous cows. For most of the physiological measurements, there was variation over the days of collection ($p<0.05$), except for haptoglobin concentration ($p>0.05$) and neutrophils: lymphocytes ratio ($p>0.05$).
**Conclusion:** Nellore heifers show physiological changes caused by abrupt weaning and are slight influenced by cow parity.

**Keywords:** *Bos indicus*, Beef cattle; Ceruloplasmin; Cortisol; Physiology.

## INTRODUCTION

The transition from the pre-weaning to the rearing phase is marked by weaning, which is a critical period in the productive life of beef calves due to the multiple stressors to which the animals are exposed such as the loss of their dam, changes in diet, and physical and social environment [1–3]. Due to these compounded stressors, calves often exhibit physiological responses, such as increased cortisol, neutrophil: lymphocyte ratio, and acute phase proteins that are associated with lower performance in the post-weaning phase [4–6].

In addition, the nutritional condition of the cow-calf pair is essential to guarantee an adequate immune response to the challenges associated with the stress during the separation [7]. Thus, natural factors of the cow, such as maternal ability and milk production, influence the performance of calves and are directly related to the cow-calf bond [8] and are identified as one of the potential influencers of stress responses [9]. Maternal ability encompasses a set of actions associated with maternal behavior, such as milk production and the ability to protect the offspring, which are related to the mother's temperament and reproductive life, so that female mammals, in general, are better dams after the second calving [10]. Furthermore, multiparous cows produce more milk than primiparous cows [11], which could influence calves' performance [12].

Ungerfeld et al. [13] presented that the behavior of 6-month-old beef calves at weaning differed according to their mothers' milk yield and developmental growth stage, which calves from cows with the highest milk yield spent less time grazing before and after weaning than calves from cows with the lowest milk yield. However, all cows used in this work were primiparous crossbred cows. Therefore, it is not possible to state whether these differences would also occur at different parities. Moreover, there is evidence that in response to the stress of abrupt weaning, multiparous cows show more marked behavioral and physiological changes when compared to primiparous cows [14,15]. However, there is a lack of information on the effect of parity on the response to stress in weaning calves.

The stress response is a complex process because it will depend on the duration and frequency of stressor stimuli. Therefore, understanding the physiological changes and the biological markers involved in the weaning stress response allows nutritionists, veterinarians, and producers to develop management strategies that improve the herd's health, productivity, and well-being. In addition, little is known whether parity can influence the intensity of nutritional and social dependence, which could lead to changes in calves' ability to adapt to weaning. Accordingly, we hypothesize that cow parity order influences the weaning stress response in Nellore heifers, in which offspring of multiparous cows show more significant physiological changes than those of secundiparous and primiparous cows. Therefore, this study aimed to evaluate the influence of the dam's parity on the magnitude of stress response to abrupt weaning of Nellore heifers under grazing.

## MATERIALS AND 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 No. 071/2019). The experiment was conducted at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20°45′S 42°52′W), between February and July 2019. The experimental period lasted 154 d and was divided into a pre-weaning phase (140 d) and a post-weaning phase (14 d).

### *Experimental design and animals*

Thirty Nellore female calves with their respective dams were used and presented the following average age and body weight (BW): 85 ± 7 days and 97 ± 18 kg, respectively. The female calves were divided according to the parity order of the dams: 10 primiparous (2 years, 451 ± 61 kg), 10 secundiparous (3 years, 492 ± 43 kg), and 10 multiparous (4–6 years, 537 ± 43 kg). The female calves used in this study were born between September and October 2018 and came from the same beef herd. Cow-calf pairs were randomly allocated to five paddocks covered with Urochloa decumbens grass (8.7 ha/paddock), where two female calves from each group were placed in the paddocks (six cow-calf pairs/paddock) 14 days before the beginning of the experiment to acclimate to the environment and social interactions. All animals were monitored for clinical signs to ensure they were healthy.

During the pre-weaning phase, female calves were group-fed (six calves/paddock) with 6 g/kg BW of an energy-protein supplement containing 300 g crude protein (CP)/kg dry matter (DM) in a creep-feeding system (Table 1). In the post-weaning phase, heifers were group-fed with 7 g/kg BW on a supplement formulated with the same ingredients to contain 200 g CP/Kg DM (Table 1). The supplements were formulated to supply approximately 50% of the CP requirement of heifers in the pre-weaning and post-weaning phases, based on the BR-Corte system [16]. The supplements were provided daily at 1100 h and were adjusted every 28 days after weighing the animals (without fasting). All animals had free access to water and a commercial mineral mix (Table 1).

Female calves were abruptly weaned (d 0) at an average age of 225 ± 7 days. They were then loaded onto a livestock truck and transported for 1.6 km to a different experimental area located at a sufficient distance away to avoid auditive interaction with the dams. Each group of heifers (six heifers/paddock) was maintained throughout the experiment, maintaining the social relationships established before weaning.

*Sampling in the pre-weaning phase*

Forage collections were carried out every 28 d, to quantify total dry matter (DM) per ha, by cutting close to the ground in five areas delimited by a metallic square (0.5 × 0.5 m), randomly selected in each experimental paddock. Also, on the same day, it was hand plucking to evaluate the forage selected by animals (chemical composition).

The average daily gain (ADG) of heifers was measured by weighing on d -140 (iBW: initial body weight) and on d -13 (fBW: final body weight) after 14 h fasting from solids.

Blood samples were collected from heifers on d -5 (at the end of the pre-weaning phase). 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 quantity triglycerides, total cholesterol, high-density lipoprotein (HDL), serum urea nitrogen (SUN), total proteins and albumin. 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 × g for 20 minutes, and triplicate serum or plasma samples were frozen immediately at −20 °C to await analysis.

On d -84, milk collections from cows were performed to estimate milk yields. For empty udders, calves were separated from their dams from 1500 h to 1745 h, when they were reunited with the dams and allowed to suckle. At 1800 h, calves were once again separated from dams until the next morning. At 0600 h the next day, cows were milked immediately after an injection of 10 IU oxytocin (10 IU/mL; Ocitovet®, Brazil) into the mammary vein, and the produced milk was weighed. The exact time when each cow was milked was recorded. Calves were separated from their mothers until the next collection at 1800 h, to obtain 24-h milk yields.

*Heifer response to weaning*

The heifers were weighed (unshrunk BW) at d -5 (pre-weaning baseline), d 0 (5 hours after weaning), d +1, d +3, d +7, and d +14 (post-weaning) at 08:00 h. Simultaneously, blood 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 cortisol, total proteins, and albumin. The BD Vacutainer® Plus tube with sodium heparin was used for the quantification of haptoglobin and ceruloplasmin. After collection, samples were centrifuged at 2200×g for 20 min. Serum and plasma were frozen immediately and kept at -20°C until they underwent analysis. Blood smears were prepared from whole blood with EDTA (BD Vacutainer® EDTA, São Paulo, Brazil) for hematological analysis.

*Laboratory analysis*

Forage and supplement samples were oven-dried at 55 °C for 72 h and ground at 1 or 2 mm in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA).

Samples ground at 1 mm were analyzed following the procedures described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; [18]) for dry matter (DM; method G-003/1), ash (method M-001/2), crude protein (CP; method N-001/2) and neutral detergent fiber corrected for ash and protein (apNDF; method F-001/2. iNDF [19] was quantified in samples ground at 2 mm using in situ incubation procedures with nonwoven textile bags (100 g/m²) for 288 h.

Blood concentrations of triglycerides (enzymatic-colorimetric test, K117), total cholesterol (enzymatic-colorimetric test, K083), HDL (enzymatic-colorimetric test, K071) urea (enzymatic-colorimetric method, Bioclin® K056 K056), total protein (colorimetric kinetic test, Bioclin® K031), albumin (bromecresol green method, Bioclin® K040), and glucose (enzymatic glucose oxidase-peroxidase method, Bioclin® K082) were quantified using an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). Serum cortisol concentrations were analyzed by the chemiluminescent method (Beckman Coulter®, 33600) using the Access® 2 analyzer system (Beckman Coulter®, Brea, USA).

The equation: TC = HDL + LDL + VLDL, where TC = total cholesterol and VLDL = triglycerides/5 [20], was used for calculating the serum contents of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL). Globulins were calculated by the difference between total proteins and albumin. SUN was estimated as 46.67% of total serum urea.

Plasma ceruloplasmin was quantified in duplicate samples by the oxidase activity method using colorimetric procedures, as described by Demetriou et al. [21]. Plasma haptoglobin concentration was quantified in duplicate samples by estimation of differences in peroxidase activity caused by haptoglobin-hemoglobin complexing [22]; the results were expressed as optical density resulting from readings at 450 nm × 100 [23].

Red blood cell (RBC) and white blood cell (WBC) count, and hemoglobin concentration (HB) were quantified using the Hematoclin 2.8 Vet automatic analyzer (Bioclin®, Belo Horizonte, Brazil), the differentiation of leukocyte cells (neutrophils and lymphocytes) were determined under a microscope, and neutrophil to lymphocyte (N:L) ratios were calculated. Hematocrit (HCT) was quantified in a microcentrifuge (MicroSpin microhematocrit centrifuge, Jaboticabal, Brazil).

*Statistical analysis*

The experiment was analyzed using the model:

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

where $Y_{ijk}$ is the observation taken on animal $k$, pertaining to parity $j$, and managed in the paddock $i$; μ is the overall constant; $P_i$ is the random effect of paddock $i$; $O_j$ is the fixed effect of parity order $j$; and $e_{(ij)k}$ is the random error, assumed to be NIID ($0, \sigma 2\ e$).

Blood parameters taken over time in the same animals were evaluated as repeated measurements, where the best structure of the (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 analysis were performed using the PROC MIXED of SAS 9.4 (Inst. Inc., Cary, NC, USA). All the statistical evaluations were performed considering 0.05 as the critical level of probability for the occurrence of the type I error.

## RESULTS

*Pre-weaning phase*

The iBW (p=0.101), fBW (p=0.201), and ADG (p=0.422) of female calves were similar between parities (Table 2). The milk yield of dams differs between parity orders, where multiparous cows produce more milk compared to secundiparous and primiparous cows (p=0.049; Table 2).

At the end of the pre-weaning phase, the concentrations of glucose (p= 0.334), triglycerides (p=0.599), total cholesterol (p=0.077), LDL (p=0.162), HDL (p=0.192), total proteins (p=0.898), albumin (p=0.621), globulins (p=0.747), and SUN (p=0.141) were not affected by the treatments (Table 3).

*Heifer response to abrupt weaning*

BW was not affected by the interaction (p=0.844) and parity (p=0.332). However, it was affected by day (p<.0001; Figure. 1). The BW of the heifers varied between 188.14 and 203.19, reaching a maximum weight at d +14 and the average minimum weight at d +3 . There was no interaction between parity and collection day for any of the physiological responses (p>0.05; Table 4). Only the cortisol concentration of the heifers was influenced by the dam's parity (p=0.040; Table 4), with higher concentration for heifers born to multiparous than primiparous cows.

For most of the physiological measurements, there was variation over the days of collection (p<0.05; Table 4), except for haptoglobin concentration (p=0.070; Table 4) and N:L ratio (p=0.922; Table 4). The concentrations of cortisol and ceruloplasmin increased soon after weaning on d 0 (5 hours later), with the highest serum cortisol concentrations of 16.30 ng/mL on d +1 and return to pre-weaning baseline of 9.95 ng/mL on d +7 (p<.0001; Figure. 2a). For ceruloplasmin, concentrations peaked on d +1 and d +7, with subsequent decrease on d +14 to the pre-weaning baseline (p<.0001; Figure. 2b). Total proteins concentration increased on d +7 and remained elevated on the last day of collection (p=0.004; Figure. 2c). Albumin levels decreased on d +3, increased on d +7, and on d +14 it appeared to be returning to its initial pre-weaning concentration (p=0.001; Figure. 2d).

Concerning RBC count, there was a decrease shortly after weaning and a further increase on the following day, and it appeared to be returning to baseline levels on d +3 (p<.0001; Figure. 3a). Also, HCT percentage (p<.001; Figure. 3b) and HB concentration (p=0.003; Figure. 3c) decreased 5 hours after separation. For HCT percentage, there was a significant increase on d +1, and a reduction on d +3. Already for HB concentration, there was an increase on d +1, and a reduction to baseline levels on d +3.

Immediately after separation (5 hours later), the heifers showed an increase in the number of WBC count (p<.0001; Figure. 4a), lymphocytes (p<.0001; Figure. 4c), and neutrophils (p=0.001; Figure. 4b), and a subsequent decrease on d +1. In addition, from d +1 for WBC and lymphocytes these counts reached lower numbers than those found at the pre-weaning baseline. For the number of neutrophils there was a return to pre-weaning levels on d +14.

## DISCUSSION

Our study provides physiological evidence confirming that weaning is one stressful time for beef calves in their productive life. However, it was not clear whether the parity order of the dams influences the stress response, despite higher cortisol concentrations for heifers from multiparous cows. With these findings alone, it was not possible to confirm whether the bond is stronger between calves and multiparous cows due to the greater milk supply and, therefore, the more disturbing the weaning process would be.

Although multiparous cows produced more milk and calves probably suckled more, a possible explanation for the non-significance in performance and metabolic characteristics evaluated in the pre-weaning phase, and in the stress response of weaning is the age at weaning, since milk is the most important factor in calf weight gain, but its importance decreases with age. For example, in Nellore, calves are more dependent on solid feed from the third month of age [24]. In addition, during early life, most social interactions of calves involve the dam, therefore, stress is more remarkable in early weaning because of the stronger cow-calf bond [25]. Likewise, in beef cows, Stěhulová et al. [9] found the strongest factor affecting the cow reaction in the weaning is the age of the calf. In fact, Ungerfeld et al. [14] showed that early weaning-induced behavioral changes were more significant in multiparous than primiparous cows.

In addition, a possible explanation for the absence of differences in performance and metabolic characteristics is that calves born from cows with lower Milk yields tend to increase forage consumption to consume similar amounts of metabolizable energy per unit of weight [26,27]. Indeed, in a previous study, it was shown that calves born from primiparous cows had more grazing time than calves born from multiparous cows [28].

On the other hand, in another experiment with the dams of these heifers, we found that under conventional weaning management (weaning at 7 to 8 months), multiparous cows exhibited marked distress reflected in more remarkable physiological changes and a trend toward a higher frequency of vocalization, compared to secundiparous and primiparous cows, notably, a higher concentration of cortisol was found on d +7 [15]. Thus, as in this study, we showed that the offspring of multiparous cows had higher cortisol concentrations when compared to the other categories. However, as this is an isolated finding, it seems that around

7.5 months the maternal-offspring bond is still strong, but parity order is a factor that may influence the stress response less in calves than in cows.

Notably, the ADG and final weight in the pre-weaning phase of the heifers is consistent with other studies with Nellore heifers supplemented under grazing [29–31]. Also, an important fact to be commented on is that supplementation could not influence the stress response, as found in a previous study by our research group [32]. In the literature, it is still not clear whether supplementation reduces the stressor effects of abrupt weaning at 7 months, although supplementation is indicated as management that reduces the stress of weaning [7,33,34], it should be noted that the studies that indicate this influence evaluated the stress response in calves early weaned, Bos taurus or Bos indicus-crossbred and, in another productive system [5,35–39]. In addition, the supply of supplementation offers a greater proportion of propionic and butyric acid in the gastrointestinal tract [40], and thus, greater stimulation in the development of the ruminal mucosa and epithelium in calves [41–43], which may favor better post-weaning adaptation.

Regardless of parity order, heifers underwent the same weight variation that can be explained by behavioral changes in the first few days after the separation, such as increased locomotion, looking for the dam, lower food, and water intake, and reduced grazing and rumination time, which are features of the weaning process in both calves and cows [15,44–48]. Previous experiments showed that feed and water deprivation stimulated the mobilization of fat reserves and activation of the hypothalamic-pituitary-adrenal axis, eliciting neuroendocrine and acute-phase protein responses [49–51].

Those explanations are supported by the increase in cortisol and ceruloplasmin concentrations that are indicated as a modulator of stress adaptation [52,53]. During a stress challenge, the acute increase in cortisol concentration is considered one of the most important physiological effects, as it generates an essential acute phase response for the animal to deal with stressors and serves as a modulator of the immune response. Furthermore, it could also be assumed that differences in blood total protein and albumin are related to the weaning process. Since, with the increase in the production of positive acute phase proteins, there is a hepatic regulation with a decrease in the synthesis of normal blood proteins such as albumin [52], which is known as a negative acute phase protein, and often used to monitor the health and welfare of animals in large herds [54].

This water deprivation, which probably occurred shortly after weaning, could also have led to a significant peak in RBC count, HCT percentage, and HB concentration on the first day after weaning, as reported in other experiments with newly weaned calves [32,55] and cows[15]. These results, combined with the increased cortisol concentration, reinforce that the heifers drank lower water after weaning. Whereas, in the presence of water deprivation, cortisol may serve to protect and maintain water balance in times of stress, because excess cortisol interferes with the mechanisms of resistance to dehydration in Bos indicus genotypes [53].

After a stressor stimulus, the homeostasis is altered at numerous levels which may affect immunocompetence in beef calves [7]. With increasing corticosteroids, more profound changes in the number and function of neutrophils are evidenced, leading to neutrophilia. In addition, corticosteroids also induce lymphopenia through lympholysis and decreased lymphocyte recirculation [56]. Thus, many studies with newly weaned calves show an increase in the N:L ratio [55,57,58]. Lynch et al. [7] showed in their review that these changes may induce a state, whereby the protective actions of these phagocytosing cells begin to damage tissue, thus, allowing opportunistic bacteria to become resident locally. Moreover, the redistribution of lymphocyte subsets may attenuate adaptive immune responses, reducing the capacity to monitor foreign antigens. Both these factors could influence the efficacy of vaccination protocols in weaned beef calves.

In this study, for the leukocyte population, the greatest values for WBC and neutrophils occurred within the first 5 hours post-weaning and already decreased the next day, which is consistent with previous findings that have indicated that increased total leukocyte number was represented by profound neutrophilia [59]. However, previous authors have reported that these increases in neutrophil numbers occurred later and remain for a few days after weaning [57,60]. For example, Lynch et al. [60], found higher values in newly weaned calves on days 2 and 7 after weaning. As well as Lomborg et al. [61] found neutrophilia in cattle 8 and 24 hours after exposure to complex stress (transportation, tie stall housing, slippery floors, and social isolation).

Interestingly, in this study, there was an unexpected increase in the number of lymphocytes since other authors reported lymphopenia [55,57,58,60] or no significant change [61] in response to the stress. In this study, this lower number of lymphocytes was observed late on d +7 but not in line with the reference limits for beef cattle [62]. Paes et al. [63], also observed higher values of lymphocyte number for Nellore calves, which can be attributed to the tropical conditions where the animals are allocated. For instance, there is greater contact of these animals with antigens, such as ectoparasites and endoparasites. Also, previously our research group carried out a study with Nellore heifers that showed high values for lymphocytes compared to the reference values [32], already in Nellore cows, the values reported for all cows were in line with the reference limits for beef cows [15].

Thus, these controversies seem to be associated with the age of the animal and not the incidence of diseases. Previously it was described that age factors have a significant influence on the WBC count of healthy Nellore females, as young calves had higher total numbers of leukocytes than adults, with a predominantly lymphocytic differential cell picture, in which the total absolute number of lymphocytes (typical and atypical) showed a gradual and significant increase in its values up to one year of life [64], elicited by the more intense immunogenic activity.

Therefore, the peak of lymphocytes accompanied by neutrophilia on the day of weaning may be a little-known characteristic of the stress leukogram since most studies did not assess physiological responses hours after weaning. Another relevant point to be considered is that this unexpected increase in lymphocytes number, despite the neutrophilia, contributed to the fact that we did not find significant differences in the N:L ratio, which would be expected in a typical stress leukogram [55,61,63,65]. Thereby, discrepancies between some studies can be attributed to differences in age, weaning method, breed, sex, parity, and management [5,7,13,36,57].

In summary, cow parity did not influence the stress response at weaning, performance, and metabolic characteristics during the pre-weaning period of Nellore heifers under grazing. Furthermore, breaking abruptly the mother–offspring bond under traditional weaning ages (7-8 months) represented a stressful experience for Nellore heifers, causing physiological changes, which were mostly observed during the first days after the separation. It should be noted that there is still difficulty in accurately quantifying the magnitude and severity of the stress response at weaning, however, it is emphasized that the levels of cortisol, acute phase proteins (ceruloplasmin and albumin) and hematological variables in the first week after weaning can be a useful tool to monitor and adapt better management on the farming Beef Cattle. In addition, the higher concentration of cortisol in heifers from multiparous cows is an important finding, since during a stress challenge, the acute increase in cortisol concentration is considered one of the most important physiological effects, as it generates an essential acute phase response for the animal to deal with stressors and serves as a modulator of the immune response [52].

## CONCLUSIONS

Nellore heifers show physiological changes caused by abrupt weaning at 7,5 months, these changes occur mainly in the first week after separation and are slight influence by cow parity.

**REFERENCES**

1. Weary, D.M.; Jasper, J.; Hötzel, M.J. Understanding Weaning Distress. Applied Animal Behaviour Science 2008, 110, 24–41, doi:10.1016/j.applanim.2007.03.025.
2. Ungerfeld, R.; Hötzel, M.J.; Quintans, G. Changes in Behaviour, Milk Production and Bodyweight in Beef Cows Subjected to Two-Step or Abrupt Weaning. Anim. Prod. Sci. 2015, 55, 1281, doi:10.1071/AN13453.
3. Orihuela, A.; Galina, C.S. Effects of Separation of Cows and Calves on Reproductive Performance and Animal Welfare in Tropical Beef Cattle. Animals 2019, 9, 223, doi:10.3390/ani9050223.
4. Qiu, X.; Arthington, J.D.; Riley, D.G.; Chase, C.C.; Phillips, W.A.; Coleman, S.W.; Olson, T.A. Genetic Effects on Acute Phase Protein Response to the Stresses of Weaning and Transportation in Beef Calves1,2. Journal of Animal Science 2007, 85, 2367–2374, doi:10.2527/jas.2006-843.
5. Arthington, J.D.; Qiu, X.; Cooke, R.F.; Vendramini, J.M.B.; Araujo, D.B.; Chase, C.C.; Coleman, S.W. Effects of Preshipping Management on Measures of Stress and Performance of Beef Steers during Feedlot Receiving1. Journal of Animal Science 2008, 86, 2016–2023, doi:10.2527/jas.2008-0968.
6. Cooke, R.F.; Arthington, J.D.; Austin, B.R.; Yelich, J.V. Effects of Acclimation to Handling on Performance, Reproductive, and Physiological Responses of Brahman-Crossbred Heifers. Journal of Animal Science 2009, 87, 3403–3412, doi:10.2527/jas.2009-1910.
7. Lynch, E.; McGee, M.; Earley, B. Weaning Management of Beef Calves with Implications for Animal Health and Welfare. Journal of Applied Animal Research 2019, 47, 167–175, doi:10.1080/09712119.2019.1594825.
8. Quintanilla, R.; Piedrafita, J. Efectos Maternos En El Peso al Destete Del Ganado Vacuno de Carne: Una Revision. ITEA Producción animal 2000, 96A(1), 7–39.
9. Stěhulová, I.; Valníčková, B.; Šárová, R.; Špinka, M. Weaning Reactions in Beef Cattle Are Adaptively Adjusted to the State of the Cow and the Calf. Journal of Animal Science 2017, 95, 1023, doi:10.2527/jas2016.1207.
10. Paranhos da Costa, M.J.R.P. da; Schmidek, A.; Luciandra M. de Toledo Relações Materno-Filiais Em Bovinos de Corte Do Nascimento à Desmama. Revista Brasileira de Reprodução Animal 2007, 31, 183–189.

11. Ferreira, M.F. de L.; Rennó, L.N.; Rodrigues, I.I.; Valadares Filho, S. de C.; Costa e Silva, L.F.; Silva, F.F. e; Detmann, E.; Paulino, M.F. Evaluation of Non-Linear Models to Predict Potential Milk Yield of Beef Cows According to Parity Order Under Grazing. Front. Vet. Sci. 2021, 8, 721792, doi:10.3389/fvets.2021.721792.
12. Mpofu, T.J.; Nephawe, K.A.; Ginindza, M.M.; Siwendu, N.A.; Mtileni, B. Cow Efficiency, Relative-Birth Weight and Subsequent Pre-Weaning Growth Performance of Nguni Cattle. American Journal of Animal and Veterinary Sciences 2022, 17, 113–121, doi:10.3844/ajavsp.2022.113.121.
13. Ungerfeld, R.; Quintans, G.; Enríquez, D.H.; Hrötzel, M.J. Behavioural Changes at Weaning in 6-Month-Old Beef Calves Reared by Cows of High or Low Milk Yield. Anim. Prod. Sci. 2009, 49, 637, doi:10.1071/AN09037.
14. Ungerfeld, R.; Hötzel, M.J.; Scarsi, A.; Quintans, G. Behavioral and Physiological Changes in Early-Weaned Multiparous and Primiparous Beef Cows. Animal 2011, 5, 1270–1275, doi:10.1017/S1751731111000334.
15. de Paula, C.; Rennó, L.N.; Martins, H.C.; Rodrigues, I.I.; Detmann, E. Does Parity Influence the Magnitude of the Stress Response of Nellore Cows at Weaning? Animals 2023, 13(8), 1321, doi:10.3390/ani13081321.
16. Valadares Filho, S. de C.; Silva, L.F.C. e; Gionbelli, M.P.; Rotta, P.P.; Marcondes, M.I.; Chizzotti, M.L.; Prados, L.F. Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE; 3rd ed.; Editora Federal de Viçosa, 2016; ISBN 978-85-8179-111-1.
17. Boggs, D.L.; Smith, E.F.; Schalles, R.R.; Brent, B.E.; Corah, L.R.; Pruitt, R.J. EFFECTS OF MILK AND FORAGE INTAKE ON CALF PERFORMANCE I. 1980.
18. Detmann, E.; Silva, L.F.C. e; Palma, M.N.N.; Gabriel Cipriano Rocha; Rodrigues, J.P.P. Métodos para análise de alimentos – National Institute of Science and Technology – Animal Science, INCT-CA; 2. ed.; Viscode do Rio Branco, MG, Brazil, 2022; ISBN 9786599512223.
19. Valente, T.N.P.; Detmann, E.; Queiroz, A.C. de; Valadares Filho, S. de C.; Gomes, D.I.; Figueiras, J.F. Evaluation of Ruminal Degradation Profiles of Forages Using Bags Made from Different Textiles. R. Bras. Zootec. 2011, 40, 2565–2573, doi:10.1590/S1516-35982011001100039.
20. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimationof the Concentrationof Low-Density LipoproteinCholesteroiln Plasma,Without Useof the PreparativeUltracentrifuge.

21. Demetriou, J.A.; Drewes, P.A.; Gin, J.B. Ceruloplasmin. In Clinical Chemistry; Cannon, D.C., Winkelman, J.W.: Harper and Row, Hagerstown, MD, 1974; pp. 857–864.
22. Makimura, S.; Suzuki, N. Quantitative Determination of Bovine Serum Haptoglobin and It Elevation in Some Inflammatory Disease. apanese Journal of Veterinary Science 1982, 44, 15–21.
23. Cooke, R.F.; Arthington, J.D. Concentrations of Haptoglobin in Bovine Plasma Determined by ELISA or a Colorimetric Method Based on Peroxidase Activity: Methods to Determine Haptoglobin in Bovine Plasma. Journal of Animal Physiology and Animal Nutrition 2013, 97, 531–536, doi:10.1111/j.1439-0396.2012.01298.x.
24. Henriques, L.T.; Valadares Filho, S. de C.; Fonseca, M.A.; Paulino, P.V.R.; Detmann, E.; Valadares, R.F.D. Avaliação de modelos não-lineares e da relação do consumo voluntário de vacas primíparas e de bezerros com a curva de lactação de vacas Nelore. R. Bras. Zootec. 2011, 40, 1287–1295, doi:10.1590/S1516-35982011000600018.
25. Enríquez, D.H.; Ungerfeld, R.; Quintans, G.; Guidoni, A.L.; Hötzel, M.J. The Effects of Alternative Weaning Methods on Behaviour in Beef Calves. Livestock Science 2010, 128, 20–27, doi:10.1016/j.livsci.2009.10.007.
26. Baker, R.D.; Le Du, Y.L.P.; Barker, J.M. Milk-Fed Calves: 1. The Effect of Milk Intake upon the Herbage Intake and Performance of Grazing Calves. J. Agric. Sci. 1976, 87, 187–196, doi:10.1017/S0021859600026745.
27. Ansotegui, R.P.; Havstad, K.M.; Wallace, J.D.; Hallford, D.M. Effects of Milk Intake on Forage Intake and Performance of Suckling Range Calves. Journal of Animal Science 1991, 69, 899, doi:10.2527/1991.693899x.
28. Rodrigues, I.I. UNIVERSIDADE FEDERAL DE VIÇOSA. 2020.
29. da Silva, A.G.; Paulino, M.F.; da Silva Amorim, L.; Rennó, L.N.; Detmann, E.; de Moura, F.H.; Manso, M.R.; Silva e Paiva, P.H.; Ortega, R.E.M.; de Melo, L.P. Performance, Endocrine, Metabolic, and Reproductive Responses of Nellore Heifers Submitted to Different Supplementation Levels Pre- and Post-Weaning. Trop Anim Health Prod 2017, 49, 707–715, doi:10.1007/s11250-017-1248-1.
30. de Almeida, D.M.; Marcondes, M.I.; Rennó, L.N.; Martins, L.S.; Marquez, D.E.C.; Villadiego, F.C.; Saldarriaga, F.V.; Ortega, R.M.; Moreno, D.P.S.; Lopes, S.A.; et al. Supplementation Strategies for Nellore Female Calves in Creep Feeding to Improve the Performance: Nutritional and Metabolic Responses. Trop Anim Health Prod 2018, 50, 1779–1785, doi:10.1007/s11250-018-1619-2.

31. de Paula, C.; Rennó, L.N.; Ferreira, M.F. de L.; Silva, Á.E.M. da; Moreira, S.S.; Assis, G.J. de F.; Detmann, E.; Valadares Filho, S. de C.; Fonseca Paulino, M.; Santos, G.M. dos Effect of Pre- and Post-Weaning Supplementation on Performance, Nutritional, and Metabolic Characteristics in Nellore Heifers under Grazing. Anim. Prod. Sci. 2022, 62, 1706–1719, doi:10.1071/AN22025.
32. de Paula, C. Nutritional plans for Nellore heifers grazing in the pre and post-weaning stages: weaning stress, performance and metabolism. dissertation, Universidade Federal de Viçosa: Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, 2019.
33. Enríquez, D.H.; Ungerfeld, R.; Quintans, G.; Guidoni, A.L.; Hötzel, M.J. The Effects of Alternative Weaning Methods on Behaviour in Beef Calves. Livestock Science 2010, 128, 20–27, doi:10.1016/j.livsci.2009.10.007.
34. Cooke, R.F. Invited Paper : Nutritional and Management Considerations for Beef Cattle Experiencing Stress-Induced Inflammation 1 1This Article Was Based on a Presentation at the ARPAS Symposium "Understanding Inflammation and Inflammatory Biomarkers to Improve Animal Performance" at the 2016 Joint Annual Meeting, July 19–23, 2016, Salt Lake City, Utah. The Professional Animal Scientist 2017, 33, 1–11, doi:10.15232/pas.2016-01573.
35. Myers, S.E.; Faulkner, D.B.; Ireland, F.A.; Berger, L.L.; Parrett, D.F. Production Systems Comparing Early Weaning to Normal Weaning with or without Creep Feeding for Beef Steers. Journal of Animal Science 1999, 77, 300, doi:10.2527/1999.772300x.
36. Blanco, M.; Villalba, D.; Ripoll, G.; Sauerwein, H.; Casasús, I. Effects of Pre-Weaning Concentrate Feeding on Calf Performance, Carcass and Meat Quality of Autumn-Born Bull Calves Weaned at 90 or 150 Days of Age. Animal 2008, 2, 779–789, doi:10.1017/S1751731108001808.
37. Cooke, R.F.; Bohnert, D.W.; Moriel, P.; Hess, B.W.; Mills, R.R. Effects of Polyunsaturated Fatty Acid Supplementation on Ruminal in Situ Forage Degradability, Performance, and Physiological Responses of Feeder Cattle1. Journal of Animal Science 2011, 89, 3677–3689, doi:10.2527/jas.2010-3515.
38. Lynch, E.M.; McGee, M.; Doyle, S.; Earley, B. Effect of Pre-Weaning Concentrate Supplementation on Peripheral Distribution of Leukocytes, Functional Activity of Neutrophils, Acute Phase Protein and Behavioural Responses of Abruptly Weaned and Housed Beef Calves. BMC Vet Res 2012, 8, 1, doi:10.1186/1746-6148-8-1.
39. Hahn, M.D. Methods to Reducing Weaning Stress in Early Weaned Spring Beef Calves. 2021.

40. Lourenco, J.M.; Kieran, T.J.; Seidel, D.S.; Glenn, T.C.; Silveira, M.F. da; Callaway, T.R.; Stewart, R.L. Comparison of the Ruminal and Fecal Microbiotas in Beef Calves Supplemented or Not with Concentrate. PLoS ONE 2020, 15, e0231533, doi:10.1371/journal.pone.0231533.

41. Sander, E.G.; Warner, R.G.; Harrison, H.N.; Loosli, J.K. The Stimulatory Effect of Sodium Butyrate and Sodium Propionate on the Development of Rumen Mucosa in the Young Calf. Journal of Dairy Science 1959, 42, 1600–1605, doi:10.3168/jds.S0022-0302(59)90772-6.

42. Nutrient Requirements of Dairy Cattle; National Research Council (U.S.), Ed.; 7th rev. ed.; National Academy Press: Washington, D.C, 2001; ISBN 978-0-309-06997-7.

43. Yáñez-Ruiz, D.R.; Abecia, L.; Newbold, C.J. Manipulating Rumen Microbiome and Fermentation through Interventions during Early Life: A Review. Front. Microbiol. 2015, 6, doi:10.3389/fmicb.2015.01133.

44. Price, E.O.; Harris, J.E.; Borgwardt, R.E.; Sween, M.L.; Connor, J.M. Fenceline Contact of Beef Calves with Their Dams at Weaning Reduces the Negative Effects of Separation on Behavior and Growth Rate1. Journal of Animal Science 2003, 81, 116–121, doi:10.2527/2003.811116x.

45. Hötzel, M.J.; Ungerfeld, R.; Quintans, G. Behavioural Responses of 6-Month-Old Beef Calves Prevented from Suckling: Influence of Dam's Milk Yield. Anim. Prod. Sci. 2010, 50, 909, doi:10.1071/AN09136.

46. Pérez-Torres, L.; Ortiz, P.; Martínez, J.F.; Orihuela, A.; Rubio, I.; Corro, M.; Galina, C.S.; Ungerfeld, R. Short- and Long-Term Effects of Temporary Early Cow–Calf Separation or Restricted Suckling on Well-Being and Performance in Zebu Cattle. animal 2021, 15, 100132, doi:10.1016/j.animal.2020.100132.

47. Pérez-Torres, L.; Orihuela, A.; Corro, M.; Rubio, I.; Alonso, M.A.; Galina, C.S. Effects of Separation Time on Behavioral and Physiological Characteristics of Brahman Cows and Their Calves. Applied Animal Behaviour Science 2016, 179, 17–22, doi:10.1016/j.applanim.2016.03.010.

48. Loberg, J.M.; Hernandez, C.E.; Thierfelder, T.; Jensen, M.B.; Berg, C.; Lidfors, L. Weaning and Separation in Two Steps—A Way to Decrease Stress in Dairy Calves Suckled by Foster Cows. Applied Animal Behaviour Science 2008, 111, 222–234, doi:10.1016/j.applanim.2007.06.011.

49. Ward, J.R.; Henricks, D.M.; Jenkins, T.C.; Bridges, W.C. Serum Hormone and Metabolite Concentrations in Fasted Young Bulls and Steers. Domestic Animal Endocrinology 1992, 9, 97–103, doi:10.1016/0739-7240(92)90023-Q.

50. Cooke, R.F.; Arthington, J.D.; Staples, C.R.; Thatcher, W.W.; Lamb, G.C. Effects of Supplement Type on Performance, Reproductive, and Physiological Responses of Brahman-Crossbred Females1. Journal of Animal Science 2007, 85, 2564–2574, doi:10.2527/jas.2006-684.

51. Marques, R.S.; Cooke, R.F.; Francisco, C.L.; Bohnert, D.W. Effects of Twenty-Four Hour Transport or Twenty-Four Hour Feed and Water Deprivation on Physiologic and Performance Responses of Feeder Cattle1. Journal of Animal Science 2012, 90, 5040–5046, doi:10.2527/jas.2012-5425.

52. Gruys, E.; Toussaint, M.J.M.; Niewold, T.A.; Koopmans, S.J. Acute Phase Reaction and Acute Phase Proteins. J Zheijang Univ Sci B 2005, 6, 1045–1056, doi:10.1631/jzus.2005.B1045.

53. Parker, A.J.; Hamlin, G.P.; Coleman, C.J.; Fitzpatrick, L.A. Excess Cortisol Interferes with a Principal Mechanism of Resistance to Dehydration in Bos Indicus Steers. 2004.

54. Paulina, J.; Tadeusz, S. Acute Phase Proteins in Cattle. In Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases; Veas, F., Ed.; InTech, 2011 ISBN 978-953-307-873-1.

55. Campistol, C.; Kattesh, H.G.; Waller, J.C.; Rawls, E.L.; Arthington, J.D.; Carroll, J.A.; Pighetti, G.M.; Saxton, A.M. Effects of Pre-Weaning Feed Supplementation and Total versus Fenceline Weaning on the Physiology and Performance of Beef Steers. Int. J. Livest. Prod. 2016, 7, 48–54, doi:10.5897/IJLP2016.0291.

56. Jain, N.C. Essentials of Veterinary Hematology; Philadelphia: Lea & Fabinger, 1993;

57. Hickey, M.C.; Drennan, M.; Earley, B. The Effect of Abrupt Weaning of Suckler Calves on the Plasma Concentrations of Cortisol, Catecholamines, Leukocytes, Acute-Phase Proteins and in Vitro Interferon-Gamma Production1. Journal of Animal Science 2003, 81, 2847–2855, doi:10.2527/2003.81112847x.

58. Lynch, E.M.; McGee, M.; Doyle, S.; Earley, B. Effect of Post-Weaning Management Practices on Physiological and Immunological Responses of Weaned Beef Calves. Irish Journal of Agricultural and Food Research 2011, 50.

59. Lynch, E.M.; Earley, B.; McGee, M.; Doyle, S. Characterisation of Physiological and Immunological Responses in Beef Cows to Abrupt Weaning and Subsequent Housing. BMC Vet Res 2010, 6, 37, doi:10.1186/1746-6148-6-37.

60. Lynch, E.M.; McGee, M.; Doyle, S.; Earley, B. Effect of Pre-Weaning Concentrate Supplementation on Peripheral Distribution of Leukocytes, Functional Activity of Neutrophils, Acute Phase Protein and Behavioural Responses of Abruptly Weaned and Housed Beef Calves. BMC Vet Res 2012, 8, 1, doi:10.1186/1746-6148-8-1.
61. Lomborg, S.R.; Nielsen, L.R.; Heegaard, P.M.H.; Jacobsen, S. Acute Phase Proteins in Cattle after Exposure to Complex Stress. Vet Res Commun 2008, 32, 575–582, doi:10.1007/s11259-008-9057-7.
62. Jones, M.L.; Allison, R.W. Evaluation of the Ruminant Complete Blood Cell Count. Veterinary Clinics of North America: Food Animal Practice 2007, 23, 377–402, doi:10.1016/j.cvfa.2007.07.002.
63. Paes, P.R. de O.; Goncalves, R.C.; Barioni, G.; Leme, F. de O.P.; Melo, M.M.; Cruz, M.L. O leucograma como indicador de estresse no desmame e no transporte rodoviário de bovinos da raça Nelore. Sem. Ci. Agr. 2012, 33, 305–312, doi:10.5433/1679-0359.2012v33n1p305.
64. Costa, J.N.; Benesi, F.J.; Birgel, E.H.; D'Angelino, J.L.; Ayres, M.C.C.; Barros Filho, I.R. de Fatores etários no leucograma de fêmeas zebuínas sadias da raça Nelore (Bos indicus). Cienc. Rural 2000, 30, 399–403, doi:10.1590/S0103-84782000000300004.
65. Earley, B.; Murray, M.; Prendiville, D.J.; Pintado, B.; Borque, C.; Canali, E. The Effect of Transport by Road and Sea on Physiology, Immunity and Behaviour of Beef Cattle. Research in Veterinary Science 2012, 92, 531–541, doi:10.1016/j.rvsc.2011.04.002.

**TABLES AND FIGURES**

**Table 1.** Proportions of ingredients in supplement base in % of dry matter, the chemical composition of supplement provided to heifers at pre-weaning and post-weaning phase and forage chemical composition.

| Variable | Supplement | | Uruchloa decumbens[5)] | | |
|---|---|---|---|---|---|
| | Pre-weaning | Post-weaning | Rainy | Rainy-dry | Dry |
| Soybean meal | 10.00 | 5.00 | - | - | |
| Corn meal | 50.00 | 50.50 | - | - | |
| Wheat bran | 38.00 | 40.00 | - | - | |
| Urea/AS[1)] | 2.00 | 2.50 | - | - | |
| Salt–mineral mix[2)] | - | 2.00 | - | - | |
| Chemical composition | | | | | |
| DM[3)] | - | - | 265.10 | 260.10 | 380.70 |
| OM[4)] | 961.90 | 949.07 | 705.70 | 920.50 | 924.20 |
| CP[4)] | 30.37 | 20.48 | 90.80 | 79.10 | 58.60 |
| apNDF[4)] | 188.35 | 252.06 | 654.80 | 682.20 | 689.30 |
| iNDF[4)] | - | - | 202.50 | 244.50 | 230.60 |

AS, ammonium sulfate; DM, dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for ash and protein; iNDF, indigestible neutral detergent fiber. [1)] Urea: ammonium sulfate (9:1). [2)] Salt–mineral mix: dicalcium phosphate (50%), common salt (47.2%), zinc sulphate (1.5%), copper sulphate (0.7%), manganese sulphate (0.5%), cobalt sulphate (0.05%), sodium selenite (0.006) and potassium iodine (0.05%). [3)] g/kg of natural matter. [4)] g/kg DM. [5)] Samples obtained by hand plucking.

**Table 2.** Performance of Nellore heifers under grazing during the pre-weaning phase and milk production of their dams according to parity order.

| Variable | Dam's parity | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Par |
| Calf iBW (Kg) | 97.21 | 87.67 | 103.80 | 6.557 | 0.101 |
| Calf fBW (Kg) | 186.76 | 178.98 | 199.61 | 9.242 | 0.201 |
| Calf ADG (Kg/d) | 0.702 | 0.724 | 0.752 | 0.033 | 0.422 |
| Cow's milk production (Kg/d) | $4.30^b$ | $4.12^b$ | $5.55^a$ | 0.476 | 0.049 |

iBW, Initial body weight; fBW, Final body weight; ADG, Average daily gain; SEM, Standard error of means; Par, Dam's Parity.

$^{a-b}$ Different letters declare significantly different between parities at $P < 0.05$.

**Table 3**. Metabolic profile of Nellore heifers under grazing during the pre-weaning phase according to parity order of their dams.

| Variable | Dam's parity | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Par |
| Glucose (mg/dL) | 86.21 | 80.67 | 85.30 | 2.98 | 0.334 |
| Triglycerides (mg/dL) | 42.02 | 37.71 | 41.52 | 3.477 | 0.599 |
| Total cholesterol (mg/dL) | 172.19 | 156.44 | 194.46 | 12.870 | 0.077 |
| LDL (mg/dL) | 76.13 | 64.63 | 93.31 | 11.746 | 0.162 |
| HDL (mg/dL) | 93.71 | 85.51 | 94.65 | 3.903 | 0.192 |
| Total proteins (g/dL) | 6.33 | 6.38 | 6.30 | 0.125 | 0.898 |
| Albumin (g/dL) | 3.13 | 3.07 | 3.14 | 0.064 | 0.621 |
| Globulins (g/dL) | 3.19 | 3.30 | 3.16 | 0.146 | 0.747 |
| SUN (mg/dL) | 14.38 | 12.43 | 12.91 | 0.885 | 0.141 |

LDL, low-density lipoprotein; HDL, high density lipoprotein; SUN, serum urea nitrogen; SEM, Standard error of means; Par, Parity.

**Table 4**. Physiological measurements in Nellore heifers under grazing at abrupt weaning according to parity order of their dams.

| Variable | Dam's parity | | | SEM | *p*-Value | | |
|---|---|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Par | Day | Par × Day |
| Cortisol (ng/mL) | 10.97[b] | 11.89[ab] | 14.02[a] | 1.157 | 0.040 | <.0001 | 0.974 |
| Ceruloplasmin (mg/dL) | 11.00 | 11.45 | 11.10 | 1.051 | 0.940 | <.0001 | 0.355 |
| Haptoglobin (OD × 100) | 3.26 | 3.39 | 3.05 | 0.124 | 0.081 | 0.070 | 0.064 |
| Total proteins (g/dL) | 6.43 | 6.54 | 6.37 | 0.061 | 0.091 | 0.004 | 0.222 |
| Albumin (g/dL) | 3.12 | 3.13 | 3.18 | 0.035 | 0.339 | 0.001 | 0.401 |
| RBC ($\times 10^6$/µl) | 10.85 | 10.28 | 10.54 | 0.315 | 0.378 | <.0001 | 0.520 |
| HCT (%) | 37.92 | 35.67 | 37.43 | 0.812 | 0.096 | <.0001 | 0.800 |
| HB (g/dL) | 11.46 | 10.78 | 11.47 | 0.266 | 0.091 | 0.003 | 0.126 |
| WBC ($\times 10^3$/µl) | 17.74 | 16.93 | 16.03 | 0.825 | 0.234 | <.0001 | 0.637 |
| Lymphocytes ($\times 10^3$/µl) | 12.19 | 11.55 | 10.76 | 0.919 | 0.434 | <.0001 | 0.509 |
| Neutrophils ($\times 10^3$/µl) | 4.52 | 4.14 | 4.38 | 0.298 | 0.612 | 0.001 | 0.071 |
| N:L | 0.40 | 0.39 | 0.44 | 0.054 | 0.712 | 0.922 | 0.208 |

OD, Optical Density; RBC, Red Blood Cell number; HCT, Hematocrit percentage; HB, Hemoglobin concentration; WBC, White Blood Cell number; N: L, Neutrophil/Lymphocyte relationship; SEM, Standard error of means; Par, Parity.

[a-b] Different letters declare significantly different between parities at $P < 0.05$.

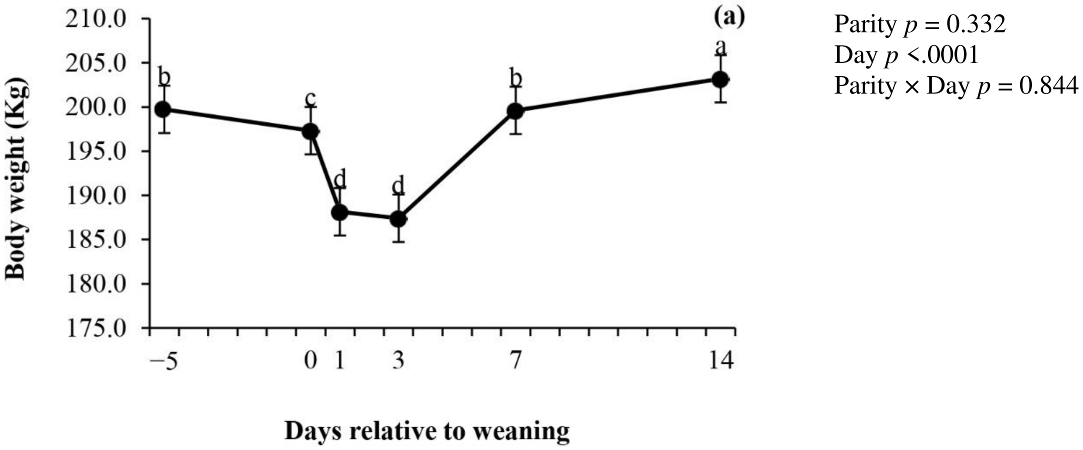
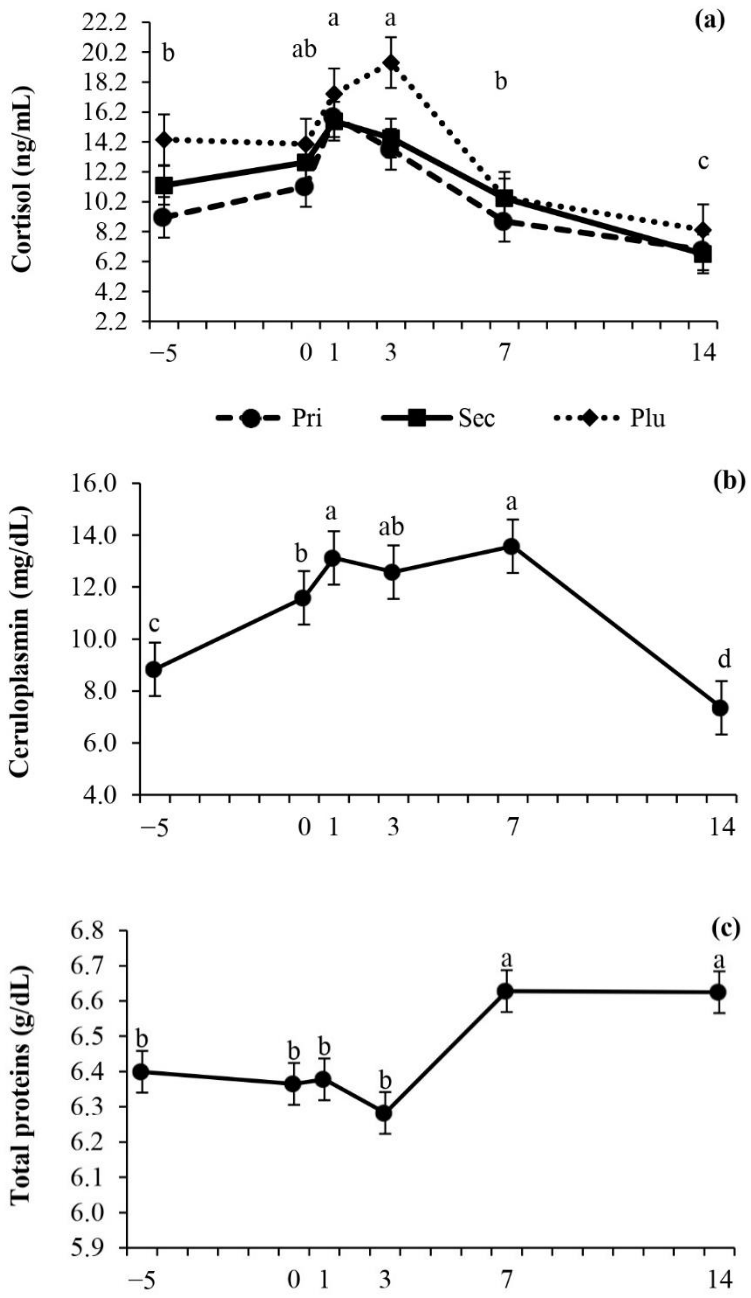



**Figure 1.** Body weight of Nellore heifers under grazing according to the days relative to abrupt weaning. Days with different superscripts differ from each other ($P < 0.05$). D 0 (5 hours after weaning).


(a)
Cortisol (ng/mL)
22.2
20.2
18.2
16.2
14.2
12.2
10.2
8.2
6.2
4.2
2.2
b
ab
a
a
b
c
−5
0
1
3
7
14
Pri
Sec
Plu
(b)
Ceruloplasmin (mg/dL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
c
b
a
ab
a
d
−5
0
1
3
7
14
(c)
Total proteins (g/dL)
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
b
b
b
b
a
a
−5
0
1
3
7
14

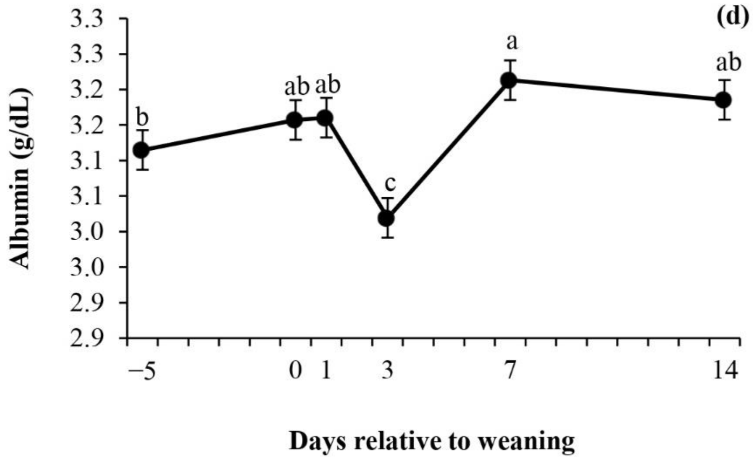



**Figure 2.** Cortisol (a), ceruloplasmin (b), total proteins (c), and albumin (d) serum concentrations in Nellore heifers under grazing according to the days relative to abrupt weaning. Days with different superscripts differ from each other ($P < 0.05$). D 0 (5 hours after weaning).

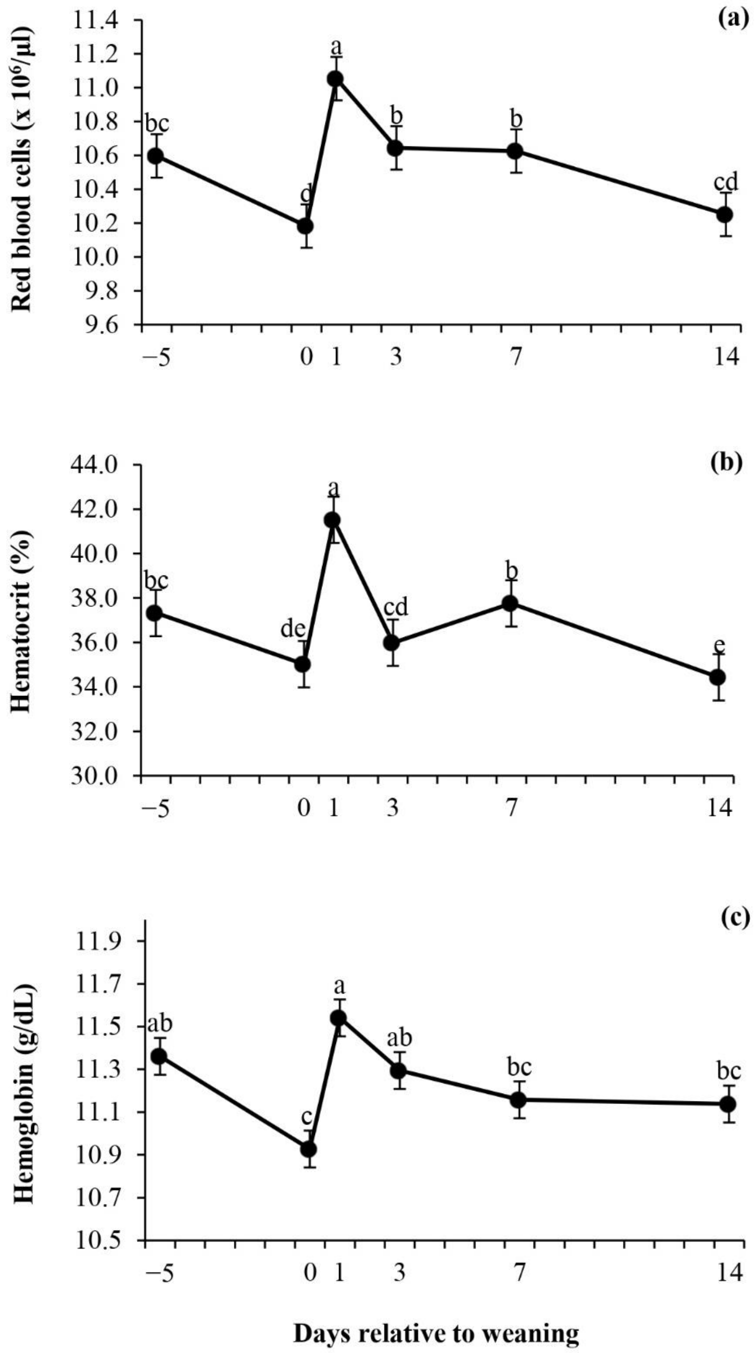



**Figure 3.** Red blood cell number (RBC; a), Hematocrit percentage (HCT; b), and hemoglobin concentration (HB; c) in Nellore heifers under grazing according to the days relative to abrupt weaning. Days with different superscripts differ from each other ($P < 0.05$). D 0 (5 hours after weaning).

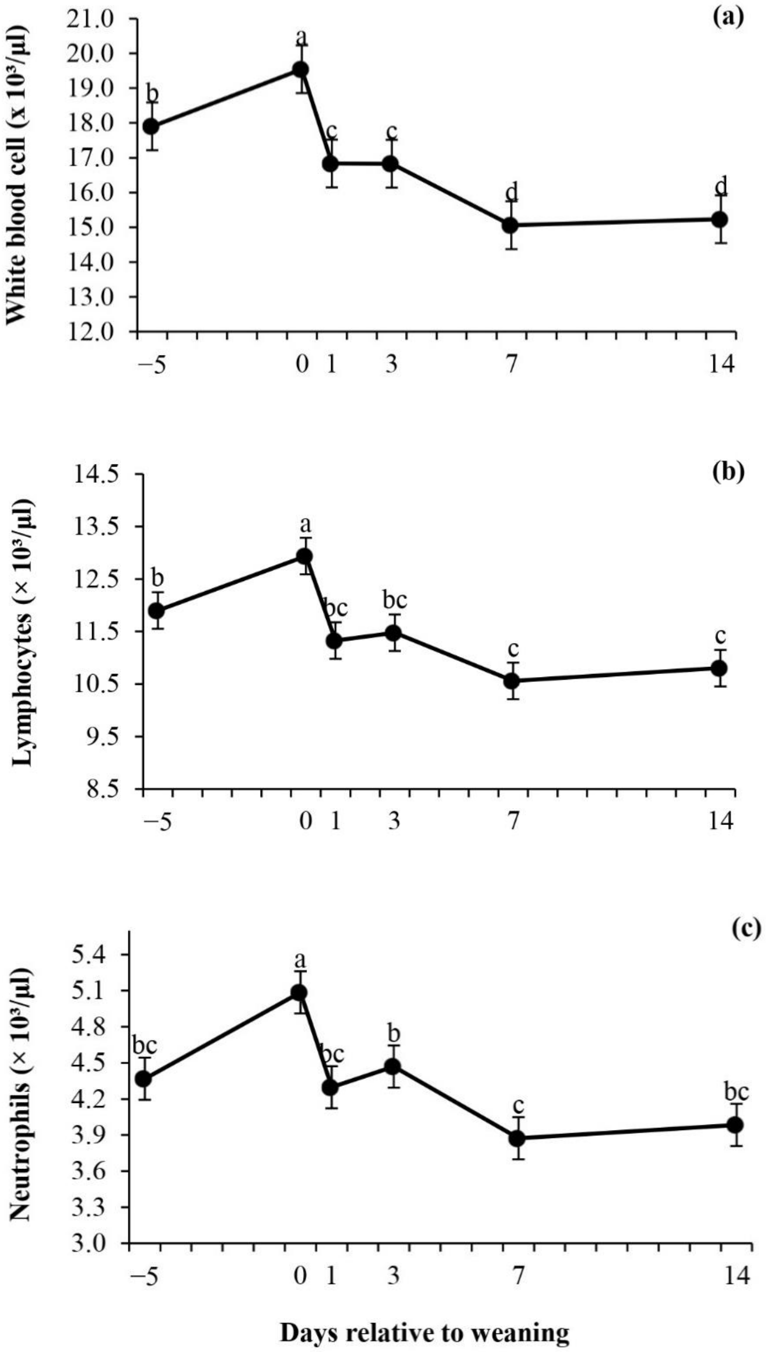



**Figure 4.** White blood cell number (WBC; a), neutrophils number (b), and lymphocytes number (c) in Nellore heifers under grazing according to the days relative to abrupt weaning. Days with different superscripts differ from each other ($P < 0.05$). D 0 (5 hours after weaning).

**CHAPTER 3 - Does dam parity influence performance, metabolic, hormonal, and reproductive characteristics in Nellore heifers under grazing in the post-weaning period?**

- In preparation to Animal Production Science

## ABSTRACT

**Context:** The nutritional status of the animal directly influences post-weaning growth characteristics, which are one of the most important factors for the intensification of the production system. Thus, parity is identified as a factor that can influence these characteristics and are related to differences in milk production and maternal ability.

**Aim:** Evaluate the influence of the dam's parity on the performance, metabolic, hormonal and reproductive characteristics of Nellore heifers under grazing in the post-weaning.

**Methods:** Thirty Nellore heifers were divided according to the parity order of the dams (10 multiparous, 10 secundiparous, and 10 primiparous), and were randomly allocated to five paddocks, where two heifers from each group were placed in the paddocks. During the post-weaning phase, data on weights, body measurements, body composition ultrasounds, and blood samples were collected from heifers.

**Key results:** Initial and final body weight and average daily gain of heifers were similar between treatments ($P > 0.05$). Body measurements were similar between heifers ($P > 0.05$), except that rump length was greater for heifers from multiparous than primiparous cows ($P < 0.05$). On d 84, the subcutaneous fat thickness of the loin and subcutaneous fat thickness on the rump were higher for heifers from multiparous cows when compared to heifers from secundiparous cows ($P < 0.05$). At the end of the experiment, all body composition measurements were similar between parities ($P > 0.05$).

In all measurements studied, the metabolic profile of heifers was similar between parities ($P > 0.05$). Except, on d 84, triglycerides and very-low density lipoprotein were higher for heifers from primiparous than heifers from secundiparous and multiparous cows ($P < 0.05$). Also, progesterone, leptin, and Insulin-like growth factor 1 concentrations were similar among heifers ($P > 0.05$). In addition, the dam's parity did not influence reproductive performance ($P>0.05$).

**Conclusions:** Maternal parity is a factor that has slight influence on the performance, metabolic, hormonal, and reproductive characteristics of Nellore heifers under pasture in the post-weaning period.

**Implications:** For replacement heifers, maternal parity has slight importance in the post-weaning growth characteristics.

**Keywords:** *Bos indicus*, beef heifers, IGF-1, growth rate, maternal effects, physiological changes, puberty, breeding season.

## INTRODUCTION

The intensification of the production system is necessary to achieve better reproductive rates, such as younger age at puberty and increasing the number of calves born per year (Vaz *et al.* 2012). In this context, it is known that the study of the growth traits of beef cattle is essential, since factors such as dam weight at calving, sex of calves, agroecological zone, the season of birth, dam age, and parity influence production traits (Mpofu et al., 2022).

The occurrence of puberty in females depends on the animal's growth rate, which provides endocrine support for controlling mechanisms of first ovulation (Silva et al., 2017). Heifers with high gain in the pre- and/ or post-weaning phases have high concentrations of hormones such as insulin-like growth factor (IGF-1) (Hiney et al. 1991; Rodríguez-Sánchez et al. 2015; de Paula et al., 2022), insulin (Adam and Findlay 1998; Almeida et al., 2019; Ortega et al. 2020), leptin (Cardoso et al. 2014), progesterone (Almeida et al., 2019) and metabolites such as glucose and free fatty acids (Garcia et al. al. 2003; Almeida et al., 2019). Furthermore, better body composition and body measurements are associated with a higher growth rate in these periods (Rodríguez-Sánchez et al. 2015; de Paula et al., 2022).

Among the maternal effects, milk production and maternal ability are identified as influencing the productive performance of beef calves from birth to age to puberty, with better results being found in calves from multiparous cows when compared to primiparous cows (Cortés-Lacruz et al., 2017). This is due to the higher milk yield of multiparous cows (Pimentel et al., 2006; Ferreira et al., 2021). Furthermore, knowing that heifer growth is limited in tropical pasture-based pasture-raising systems in periods of lower availability of good quality forages and not associated with supplementation (de Paula et al., 2022), better performance may be even more dependent on the dam's milk yield.

The effects of the dam's parity on the characteristics related to the growth rate in heifers are little studied and even less in the post-weaning phase. Therefore, our hypothesis is that Nellore heifers from multiparous cows have better performance, and metabolic, hormonal, and reproductive characteristics in the post-weaning phase. This study aimed to evaluate the influence of the dam's parity order on the performance, metabolic, hormonal and reproductive characteristics of Nellore heifers under grazing in the post-weaning.

## MATERIAL AND 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 No. 0115/2019). The experiment was conducted at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20°45′S 42°52′W), between July and December 2019. The experimental period lasted 146 d.

*Experimental design and animals*

Thirty Nellore heifers were used and presented the following average age and body weight (BW): 255 ± 7 days and 203 ± 12 kg, respectively. The heifers were divided according to the parity order of the dams: 10 primiparous (2 years, 451 ± 61 kg), 10 secundiparous (3 years, 492 ± 43 kg), and 10 multiparous (4–6 years, 537 ± 43 kg). The heifers used in this study were born between September and October 2018 and came from the same beef herd. Heifers were randomly allocated to five paddocks covered with *Urochloa decumbens* grass (8.7 ha/paddock), where two heifers from each group were placed in the paddocks (six heifers/paddock) 14 days before the beginning of the experiment to acclimate to the environment and social interactions. All animals were monitored for clinical signs to ensure they were healthy.

During the post-weaning phase, heifers were group-fed with 7 g/kg BW of an energy-protein supplement 200 g crude protein (CP)/kg dry matter (DM) (Table 1). The supplements were formulated to supply approximately 50% of the CP requirement of heifers in the post-weaning phases, based on the BR-Corte system (Valadares Filho et al. 2016). The supplements were provided daily at 11:00 h and were adjusted every 28 days after weighing the animals (without fasting). All animals had free access to water and a commercial mineral mix (Table 1).

*Sample collection*

Forage collections were carried out every 28 d, to quantify total DM per ha, by cutting close to the ground in five areas delimited by a metallic square (0.5 × 0.5 m), randomly selected in each experimental paddock. Also, on the same day, it was hand plucking to evaluate the forage selected by animals (chemical composition; Table 1).

Average daily gain (ADG) of heifers was measured by weighing at the beginning (iBW: initial body weight) and d 146 (fBW: final body weight) after 14 h fasting from solids. In addition, the weight (unshrunk BW) was measured on the day of the beginning of the timed artificial insemination (TAI) protocol (TAI BW: timed artificial insemination body weight).

Body measurements were recorded with a height stick on d 146 as follows: the height at withers (from the highest point of the shoulder blade to the ground), body length (from the anterior point of the scapulae vertically to the posterior midline), rump length (from the ischial tuberosity to the iliac tuberosity), and rump height (from the iliac tuberosity vertically to the ground). The heart girth (the body circumference immediately posterior to the front legs) were measured with a flexible tape.

Body compositions were measured using ultrasound (Aloka SSD 500; 3.5 MHz linear probe) on d 84 (at the end of the post-weaning dry period) and d 146 (at the end of the post-weaning phase). The Longissimus dorsi muscle area (LMA), loin depth and subcutaneous fat thickness of the loin (SFTL) were measured between the 12th and 13th ribs, and subcutaneous fat thickness on the rump (SFTR) between the ischium and pubis.

Blood samples were collected from heifers on d 84, d 127 (at the day of puberty induction protocol) and d 146. 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 quantity triglycerides, total cholesterol, high-density lipoprotein (HDL), serum urea nitrogen (SUN), total proteins, albumin, IGF-1 (only d 146) and leptin (only d 146). Also, a tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantify the plasma glucose concentration on the same days. After collection, samples were centrifuged at 2200 × g for 20 minutes, and triplicate samples of serum or plasma were frozen immediately at −20 °C to await analysis.

Blood samples were also collected on d 127 and d 146, with clot activator and gel for serum separation (BD Vacutainer® SST II Plus, São Paulo, Brazil), for analysis of circulating progesterone (P4) concentration, used as a reproductive indicator for the onset of puberty. A transrectal evaluation was performed, on the same days as the blood samples, using an ultrasound machine (Mindray DP2200, 7.5 mHz with linear rectal transducer, Mindray, China) to identify the presence of the corpus luteum (CL).

On d 127, all heifers were assigned to a protocol designed to increase the number of heifers presenting a CL (Sá Filho et al., 2015). The protocol consisted of the insertion of a third use P4 intravaginal insert (controlled internal drug release device [CIDR®], Zoetis, São Paulo, Brazil), plus a 2 mg estradiol benzoate (EB) injection (IM, Estrogin®, Farmavet, São Paulo, Brazil). The P4 insert was removed after nine days, and 1 mg EB was administered. On the last day of the experiment, corresponding to 10 days after P4 insert removal, blood was collected for P4 analysis, and a transrectal ultrasound evaluation was performed as described above to identify animals that had responded to the puberty induction protocol with the presence of CL.

*Laboratory analysis*

Forage and supplement samples were oven-dried at 55 °C for 72 h and ground at 1 or 2 mm in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA).

Samples ground at 1 mm were analyzed following the procedures described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al. 2022) for dry matter (DM; method G-003/1), ash (method M-001/2), crude protein (CP; method N-001/2) and neutral detergent fiber corrected for ash and protein (apNDF; method F-001/2). iNDF (Valente et al. 2011) was quantified in samples ground at 2 mm using in situ incubation procedures with nonwoven textile bags (100 g/m²) for 288 h.

Blood concentrations of triglycerides (enzymatic-colorimetric test, K117), total cholesterol (enzymatic-colorimetric test, K083), HDL (enzymatic-colorimetric test, K071) urea (enzymatic-colorimetric method, Bioclin® K056 K056), total protein (colorimetric kinetic test, Bioclin® K031), albumin (bromecresol green method, Bioclin® K040), and glucose (enzymatic glucose oxidase-peroxidase method, Bioclin® K082) were quantified using an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). P4 and leptin were analyzed by radioimmunoassay method. IGF-1 levels were quantified with DiaSorin® kits (California, USA) using an automated chemiluminescence analyzer (Liaison®, Saluggia, Italy).

The equation: TC = HDL + LDL + VLDL, where TC = total cholesterol and VLDL = triglycerides/5, were used for calculating the serum contents of low-density lipoprotein (LDL) and very-low density lipoprotein (VLDL). Globulins were calculated by the difference between total proteins and albumin. SUN were estimated as 46.67% of total serum urea.

*Statistical analysis*

The experiment was analyzed using the model:

$$Y_{ijk} = \mu + Pi + Oj + e(ij)k$$

where $Y_{ijk}$ is the observation taken on animal k, pertaining to parity j, and managed in the paddock i; μ is the overall constant; $P_i$ is the random effect of paddock i; $O_j$ is the fixed effect of parity order j; and $e_{(ij)k}$ is the random error, assumed to be NIID (0, $\sigma^2_e$).

Means were compared by Fisher's least significant difference. The degrees of freedom were estimated using the Kenward-Roger method.

The analysis was performed using the PROC MIXED of SAS 9.4 (Inst. Inc., Cary, NC, USA). 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 iBW (P = 0.225), fBW (P = 0.549), IATF BW (P = 0.649) and, ADG (P = 0.458) of heifers were similar between parities (Table 2). All Heifers had similar height at withers (P = 0.275), body length (P = 0.334), rump height (P = 0.448), heart girth (P = 0.791), and BW: height at withers ratio (P = 0.551) at the end of the experiment (Table 2). However, heifers from multiparous cows had a rump length greater than heifers from primiparous cows (P = 0.045).

On d 84, LMA (P = 0.611), and loin depth (P = 0.228) were similar between the heifers of different parities. Although, SFTL (P = 0.022) and SFTR (P = 0.049) were higher for heifers from multiparous cows when compared to heifers from secundiparous cows. At the end of the experiment, all body composition measurements were similar between parities (P > 0.05; Table 3).

In all measurements studied, the metabolic profile of heifers was similar between parities (P > 0.05; Table 4). Except, on d 84, triglycerides (P = 0.0003) and VLDL (P = 0.0003) were higher for heifers from primiparous than heifers from secundiparous and multiparous cows. Also, progesterone, leptin, and IGF-1 concentrations were similar among heifers of different parities (P > 0.05; Table 4).

In addition, 47 % of the heifers were found to have responded to the puberty induction protocol, corresponding to 14 of 30 heifers with a CL. However, the dam's parity did not influence reproductive performance, since the response to induction (P = 0.365) and the pregnancy rate at 30 (P = 0.757) and 60 (P = 0.200) days after artificial insemination were similar among heifers (data not shown).

## DISCUSSION

Our study is one of the few that evaluated the effects of maternal parity on growth characteristics in post-weaning Nellore heifers, showing that there is a relationship between higher parity of the cow and better performance of her offspring, however, this factor affects weakly the performance, hormonal and metabolic characteristics and did not influence the reproductive characteristics.

Authors have shown that the maternal effects that most influence the performance of calves are the maternal ability to protect the calf and milk yield (Quintanilla and Piedrafita 2000). In addition, it is shown that there is a relationship between higher parity and higher milk yield that affects the growth ratios of their calves during the pre-weaning phase, and that can also influence the higher weight gain during the post-weaning phase (Cortés-Lacruz et al. 2017). In fact, in a previous study with the dams, we found that multiparous produced more milk than primiparous cows, however, the same female calves did not perform better in the pre-weaning phase (de Paula et al., unpublish data).

In another study, the authors showed that the lower milk yield of primiparous cows was because these animals were still growing, and this was reflected in more unbalanced metabolic and hormonal characteristics and a reduced body condition score in the pre- and postpartum. Nonetheless, despite this higher milk supply for calves from multiparous cows, there was no greater weaning weight gain when compared to calves from primiparous cows (Ferreira et al. 2021).

At the genetic level, has been shown that weaning weight depends on both the genetic potential of the calf (direct effect) and the effect of the dam (maternal effect), also, genetic milk yield explains half the variation of maternal effects (Cortés-Lacruz et al. 2017). Since calves born from primiparous cows spent more grazing time than offspring from multiparous cows (Rodrigues 2020), it was justified that when the milk supplement is lower, calves consume more solid feed to meet the same amount of energy needed for good growth (Baker et al. 1976; Ansotegui et al. 1991).

Cortés-Lacruz et al. (2017) showed that calves born from cows with more than two calvings had higher body weight, weight at 150 days, and mature weight than calves from secundiparous and primiparous cows. In this study, the estimates of the genetic correlation between body weight and mature weight were moderately high and positive. This result was attributed to the fact that mature weight was an estimate of the cow's growth curve; therefore, some environmental effects were not considered in the theoretical function. However, they were similar to those observed by Liinamo et al. (2001). In addition, at later ages, it is more important to consider the calf's growth capacity based on forage and concentrate intake. Since the maternal genetic effect on younger weight (90 days) is more related to milk production than the maternal genetic effect on near-weaning weight (150 days) (Cortés-Lacruz et al. 2017). Although age of dam effects on postweaning weights are generally considered important, their importance is observed to decline as the animal grows (Goldberg and Ravagnolo 2015). This can be explained as a result of compensatory growth during the post-weaning phases, being related to pre-weaning maternal effects (Brown et al. 1972). Therefore, when jointly analysing the weight gains in the pre- and post-weaning phases of the heifers in our study, we clearly see that parity cannot be correlated with weight at weaning (unpublishing date) and at 14 months of age in Nellore heifers.

The concentration of hormones, such as IGF-I and leptin, and metabolites are associated with the performance of heifers, therefore, the absence of differences in these concentrations is befitting. Although a specific analysis was not carried out to determine the consumption of these heifers, we can infer that this was similar, since, for example, the concentration of IGF-1, which is often associated with nutrient absorption and weight gain, was similar between treatments (Rodríguez-Sánchez et al. 2015; de Paula et al. 2022).

Overall, body measurements and body composition suggest that adipose and muscle tissue deposition was similar between heifers. Although, at the end of the experiment, the rump length measurement was greater for heifers born from multiparous cows compared to heifers from primiparous cows. Noteworthy that the SFTL and SFTR measurements were higher for heifers from multiparous cows when compared to heifers from secundiparous cows, only in the dry period, showing that at the end of the post-weaning phase, after an improvement in the quality of the pastures, and probably the weight gain, the heifers reached similar measures. This may also explain the absence of differences in leptin concentration at the end of the post-weaning phase. In addition, another important point to be highlighted is that all heifers had a similar response to induction, with increases in progesterone concentration of about 11%, when compared to the collection before the induction protocol. Since body reserves are related to reproductive performance, acting as an important indicator of available energy for reproductive activity (Hall et al. 1995), they may explain the similar reproductive response between treatments.

Animals are sensitive to the environment and important traits are influenced by the production environment (Mpofu et al., 2022). In fact, in a previous experiment, de Paula et al. (2022) showed that heifers with good performance, body composition, body, metabolic and hormonal measures at the end of the post-weaning phase, presented similar responses to the induction protocol and reproductive. Once again, it observed that in the dry period of the rearing phase, there are differences in the performance of heifers, but these can be reduced as long as better management conditions are achieved, aiming at a better growth rate.

In summary, our study outlined a complete panoramic profile of the main characteristics that are related to the growth of heifers, showing that parity is a factor that has slight influence on the productive and reproductive performance of Nellore heifers under grazing. These findings are important for the production system, since changes in heifer management are not necessary, which could lead to higher production costs, if the necessary gain rates for the good performance of the heifers are achieved.

## CONCLUSIONS

Nellore heifers under grazing show similar performance, metabolic, hormonal, and reproductive characteristics in the post-weaning. In addition, dam parity had slight influence on heifers' growth characteristics.

**REFERENCES**

Adam CL, Findlay PA (2001) Inhibition of Luteinizing Hormone Secretion and Expression of c-fos and Corticotrophin-Releasing Factor Genes in the Paraventricular Nucleus During Insulin-Induced Hypoglycaemia in Sheep: Inhibition of Luteinizing Hormone Secretion and Expression of c-. Journal of Neuroendocrinology 10, 777–784. doi:10.1046/j.1365-2826.1998.00263.x.

de Almeida DM, Marcondes MI, Rennó LN, Pereira Silva LH, Martins LS, Marquez DEC, Castaño Villadiego FA, Velez Saldarriaga F, Franco JDC, Moreno DPS, de Moura FH, Paulino MF (2019) Nutritional planning for Nellore heifers post-weaning to conception at 15 months of age: performance and nutritional, metabolic, and reproductive responses. Tropical Animal Health and Production 51, 79–87. doi:10.1007/s11250-018-1662-z.

Ansotegui RP, Havstad KM, Wallace JD, Hallford DM (1991) Effects of milk intake on forage intake and performance of suckling range calves. Journal of Animal Science 69, 899. doi:10.2527/1991.693899x.

Baker RD, Le Du YLP, Barker JM (1976) Milk-fed calves: 1. The effect of milk intake upon the herbage intake and performance of grazing calves. The Journal of Agricultural Science 87, 187–196. doi:10.1017/S0021859600026745.

Brown JE, Brown CJ, Butts WT (1972) A discussion of the genetic aspects of weight, mature weight and rate of maturing in hereford and angus cattle.

Cardoso RC, Alves BRC, Prezotto LD, Thorson JF, Tedeschi LO, Keisler DH, Park CS, Amstalden M, Williams GL (2014) Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers1. Journal of Animal Science 92, 2942–2949. doi:10.2527/jas.2014-7713.

Cortés-Lacruz X, Casasús I, Revilla R, Sanz A, Blanco M, Villalba D (2017) The milk yield of dams and its relation to direct and maternal genetic components of weaning weight in beef cattle. Livestock Science 202, 143–149. doi:10.1016/j.livsci.2017.05.025.

Detmann E, Silva LFC e, Palma MNN, Gabriel Cipriano Rocha, Rodrigues JPP (2022) ‘Métodos para análise de alimentos – National Institute of Science and Technology – Animal Science, INCT-CA.’ (Viscode do Rio Branco, MG, Brazil)

Ferreira MF de L, Rennó LN, Rodrigues II, Detmann E, Paulino MF, de Campos Valadares Filho S, Martins HC, Moreira SS, de Lana DS (2021) Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods. BMC Veterinary Research 17, 311. doi:10.1186/s12917-021-03019-0.

Garcia MR, Amstalden M, Morrison CD, Keisler DH, Williams GL (2003) Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age1. Journal of Animal Science 81, 261–268. doi:10.2527/2003.811261x.

Goldberg V, Ravagnolo O (2015) Description of the growth curve for Angus pasture-fed cows under extensive systems1. Journal of Animal Science 93, 4285–4290. doi:10.2527/jas.2015-9208.

Hall JB, Staigmiller RB, Bellows RA, Short RE, Moseley WM, Bellows SE (1995) Body composition and metabolic profiles associated with puberty in beef heifers1. Journal of Animal Science 73, 3409–3420. doi:10.2527/1995.73113409x.

Hiney JK, Ojeda SR, Dees WL (1991) Insulin-Like Growth Factor I: A Possible Metabolic Signal Involved in the Regulation of Female Puberty. Neuroendocrinology 420–423.

Liinamo A-E, Ojala M, Arendonk JV (2001) Genetic relationship of meat and milk production in Finnish Ayrshire. Livestock Production Science 69, 1–8. doi:10.1016/S0301-6226(00)00252-9.

Mpofu TJ, Nephawe KA, Ginindza MM, Siwendu NA, Mtileni B (2022) Cow Efficiency, Relative-Birth Weight and Subsequent Pre-Weaning Growth Performance of Nguni Cattle. American Journal of Animal and Veterinary Sciences 17, 113–121. doi:10.3844/ajavsp.2022.113.121.

Ortega RM, Paulino MF, Detmann E, Rennó LN, Moreno DS, Márquez DC, de Almeida DM, de Melo LP, Moura FH (2020) Nutritional strategies for heifers under grazing system: productive and nutritional performance, metabolic profile and ovarian activity. Tropical Animal Health and Production 52, 1013–1022. doi:10.1007/s11250-019-02095-7.

de Paula C, Rennó LN, Martins HC, Rodrigues II, Detmann E (2023) Does Parity Influence the Magnitude of the Stress Response of Nellore Cows at Weaning? doi:10.3390/ani13081321.

de Paula C, Rennó LN, Ferreira MF de L, Silva ÁEM da, Moreira SS, Assis GJ de F, Detmann E, Valadares Filho S de C, Fonseca Paulino M, Santos GM dos (2022) Effect of pre- and post-weaning supplementation on performance, nutritional, and metabolic characteristics in Nellore heifers under grazing (A Bach, Ed.). Animal Production Science 62, 1706–1719. doi:10.1071/AN22025.

Pimentel MA, Moraes JCF, Jaume CM, Lemes JS, Brauner CC (2006) Características da lactação de vacas Hereford criadas em um sistema de produção extensivo na região da campanha do Rio Grande do Sul. Revista Brasileira de Zootecnia 35, 159–168. doi:10.1590/S1516-35982006000100021.

Quintanilla R, Piedrafita J (2000) Efectos maternos en el peso al destete del ganado vacuno de carne: una revision. ITEA Producción Animal 96A(1), 7–39.

Rodrigues II (2020) Comportamento pré e pós-parto de fêmeas Nelore de diferentes ordens de parto e suas bezerras em pastejo. Dissertation in Animal Science, Universidade Federal de Viçosa, Minas Gerais, Brazil. https://locus.ufv.br//handle/123456789/29396.

Rodríguez-Sánchez JA, Sanz A, Tamanini C, Casasús I (2015) Metabolic, endocrine, and reproductive responses of beef heifers submitted to different growth strategies during the lactation and rearing periods1. Journal of Animal Science 93, 3871–3885. doi:10.2527/jas.2015-8994.

Sá Filho MF, Nasser LFT, Penteado L, Prestes R, Marques MO, Freitas BG, Monteiro BM, Ferreira RM, Gimenes LU, Baruselli PS (2015) Impact of progesterone and estradiol treatment before the onset of the breeding period on reproductive performance of Bos indicus beef heifers. Animal Reproduction Science 160, 30–39. doi:10.1016/j.anireprosci.2015.06.024.

da Silva AG, Paulino MF, da Silva Amorim L, Rennó LN, Detmann E, de Moura FH, Manso MR, Silva e Paiva PH, Ortega REM, de Melo LP (2017) Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre- and post-weaning. Tropical Animal Health and Production 49, 707–715. doi:10.1007/s11250-017-1248-1.

Valadares Filho S de C, Silva LFC e, Gionbelli MP, Rotta PP, Marcondes MI, Chizzotti ML, Prados LF (2016) ‘Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE.’ (Editora Federal de Viçosa) doi:10.5935/978-85-8179-111-1.2016B001.

Valente TNP, Detmann E, Queiroz AC de, Valadares Filho S de C, Gomes DI, Figueiras JF (2011) Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Revista Brasileira de Zootecnia 40, 2565–2573. doi:10.1590/S1516-35982011001100039.

Vaz RZ, Restle J, Pacheco PS, Vaz FN, Pascoal LL, Vaz MB (2012) Ganho de peso pré e pós-desmame no desempenho reprodutivo de novilhas de corte aos quatorze meses de idade. Ciência Animal Brasileira 13, 272–281. doi:10.5216/cab.v13i3.17527.

**TABLES**

**Table 1.** Proportions of ingredients in supplement base in % of dry matter, the chemical composition of supplement provided to heifers at post-weaning period and forage chemical composition.

| Item | Supplement | | Uruchloa decumbens | |
|---|---|---|---|---|
| | Post-weaning dry | Post-weaning dry-rainy | Dry | Dry-rainy |
| Soybean meal | 5.00 | 22.00 | | - |
| Corn meal | 50.50 | 65.00 | | - |
| Wheat bran | 40.00 | 10.00 | | - |
| Urea/AS | 2.50 | 1.00 | | - |
| Salt–mineral mix | 2.00 | 2.00 | | - |
| Chemical composition | | | | |
| DM | - | - | 380.70 | 246.00 |
| OM | 949.07 | 953.11 | 924.20 | 911.70 |
| CP | 20.48 | 20.94 | 58.60 | 121.20 |
| apNDF | 252.06 | 146.29 | 674.50 | 561.10 |
| iNDF | - | - | 230.60 | 155.20 |

Abbreviations: AS = ammonium sulfate; DM = dry matter; OM = organic matter; CP = crude protein; apNDF = detergent fiber corrected for ash and protein; iNDF = neutral detergent fiber.

[a] Urea: ammonium sulfate (9:1). [b] Salt–mineral mix: dicalcium phosphate (50%), common salt (47.2%), zinc sulphate (1.5%), copper sulphate (0.7%), manganese sulphate (0.5%), cobalt sulphate (0.05%), sodium selenite (0.006) and potassium iodine (0.05%). [C] g/kg of natural matter. [d] g/kg DM. [e] Samples obtained by hand plucking.

**Table 2**. Performance and body measurements of Nellore heifers under grazing during the post-weaning period according to parity order of their dams.

| Item | Dam's parity | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Parity |
| Post-weaning period | | | | | |
| iBW (Kg) | 198.78 | 192.64 | 212.82 | 9.041 | 0.225 |
| fBW (Kg) | 267.77 | 262.68 | 276.72 | 10.122 | 0.549 |
| TAI BW (Kg) | 298.07 | 296.67 | 308.96 | 11.214 | 0.649 |
| ADG (Kg/day) | 0.474 | 0.477 | 0.440 | 0.026 | 0.4581 |
| Height at withers | 122.41 | 124.70 | 124.00 | 1.253 | 0.275 |
| Body length | 119.49 | 117.46 | 121.20 | 1.985 | 0.334 |
| Rump length | 36.08b | 37.03ab | 39.10a | 1.020 | 0.045 |
| Rump height | 130.18 | 131.50 | 133.10 | 2.026 | 0.448 |
| Heart girth | 153.33 | 153.45 | 154.80 | 2.246 | 0.791 |
| BW:Height at withers (kg/cm) | 2.19 | 2.10 | 2.19 | 0.070 | 0.551 |

Abbreviations: iBW = Initial body weight; fBW = Final body weight; TAI BW = Timed artificial insemination body weight; ADG = Average daily gain; BW: Height at withers ratio = Body weight: Height at withers ratio; SEM = Standard error of means.

[a-b] Different letters declare significantly different between parities at $P < 0.05$.

**Table 3.** Body composition of Nellore heifers under grazing during the post-weaning period according to parity order of their dams.

| Item | Dam's parity | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Parity |
| Post-weaning dry period | | | | | |
| LMA (cm²) | 34.13 | 32.45 | 34.47 | 1.759 | 0.611 |
| Loin Depth | 58.94 | 57.65 | 61.30 | 1.711 | 0.228 |
| SFTL (mm) | 1.12ab | 0.81b | 1.41a | 0.159 | 0.022 |
| SFTR (mm) | 1.74ab | 1.42b | 1.94a | 0.157 | 0.049 |
| Post-weaning dry-to-rainy transition period | | | | | |
| LMA (cm²) | 40.75 | 42.03 | 40.92 | 1.165 | 0.623 |
| Loin Depth | 67.58 | 67.49 | 68.88 | 1.745 | 0.760 |
| SFTL (mm) | 1.52 | 1.49 | 1.73 | 0.145 | 0.319 |
| SFTR (mm) | 2.28 | 2.01 | 2.40 | 0.152 | 0.157 |

Abbreviations: LMA = Longissimus dorsi muscle area; SFTL = Subcutaneous fat thickness of the loin; SFTR = subcutaneous fat thickness on the rump; SEM = Standard error of means.
[a-b] Different letters declare significantly different between parities at $P < 0.05$.

**Table 4.** Physiological measurements in Nellore heifers under grazing during the post-weaning period according to parity order of their dams.

| Item | Dam's parity | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Primiparous | Secundiparous | Multiparous | | Parity |
| Post-weaning dry period | | | | | |
| Glucose (mg/dL) | 74.70 | 70.92 | 72.75 | 2.540 | 0.362 |
| Triglycerides (mg/dL) | 42.07a | 33.38b | 28.32b | 2.751 | 0.0003 |
| Total cholesterol (mg/dL) | 123.54 | 118.51 | 128.78 | 6.815 | 0.475 |
| VLDL (mg/dL) | 8.41a | 6.68b | 5.66b | 0.550 | 0.0003 |
| LDL (mg/dL) | 37.91 | 38.34 | 46.19 | 7.450 | 0.523 |
| HDL (mg/d) | 76.97 | 73.53 | 76.20 | 4.071 | 0.641 |
| Total protein (g/dL) | 6.29 | 6.51 | 6.38 | 0.147 | 0.461 |
| Albumin (g/dL) | 3.21 | 3.16 | 3.19 | 0.090 | 0.917 |
| Globulins (g/dL) | 3.09 | 3.35 | 3.19 | 0.141 | 0.342 |
| SUN (mg/dL) | 14.26 | 14.58 | 15.40 | 1.390 | 0.593 |
| Post-weaning dry-to-rainy transition period* | | | | | |
| Glucose (mg/dL) | 79.43 | 77.69 | 76.10 | 2.201 | 0.418 |
| Triglycerides (mg/dL) | 35.00 | 38.01 | 33.10 | 3.973 | 0.631 |
| Total cholesterol (mg/dL) | 104.28 | 94.28 | 104.02 | 6.183 | 0.393 |
| VLDL (mg/dL) | 7.00 | 7.60 | 6.62 | 0.795 | 0.631 |
| LDL (mg/dL) | 21.05 | 22.64 | 25.01 | 4.634 | 0.750 |
| HDL (mg/d) | 76.03 | 66.43 | 71.95 | 3.739 | 0.066 |
| Total protein (g/dL) | 6.36 | 6.62 | 6.43 | 0.124 | 0.185 |
| Albumin (g/dL) | 3.08 | 3.09 | 3.08 | 0.067 | 0.978 |
| Globulins (g/dL) | 3.29 | 3.52 | 3.36 | 0.120 | 0.270 |
| SUN (mg/dL) | 12.46 | 13.12 | 12.58 | 0.914 | 0.740 |
| Progesterone (ng/mL) | 0.039 | 0.048 | 0.054 | 0.014 | 0.436 |
| Post-weaning dry-to-rainy transition period | | | | | |
| Glucose (mg/dL) | 81.20 | 76.62 | 75.98 | 2.657 | 0.198 |
| Triglycerides (mg/dL) | 35.53 | 31.27 | 30.11 | 2.718 | 0.186 |
| Total cholesterol (mg/dL) | 106.06 | 92.08 | 101.36 | 6.619 | 0.274 |
| VLDL (mg/dL) | 7.11 | 6.25 | 6.02 | 0.544 | 0.186 |
| LDL (mg/dL) | 20.86 | 20.39 | 22.03 | 5.231 | 0.960 |
| HDL (mg/d) | 77.77 | 67.23 | 73.46 | 3.295 | 0.071 |
| Total protein (g/dL) | 6.51 | 6.74 | 6.46 | 0.133 | 0.209 |
| Albumin (g/dL) | 3.02 | 3.01 | 3.01 | 0.081 | 0.981 |
| Globulins (g/dL) | 3.49 | 3.74 | 3.44 | 0.127 | 0.115 |
| SUN (mg/dL) | 10.71 | 10.90 | 10.78 | 0.895 | 0.977 |
| Progesterone (ng/mL) | 0.43 | 0.44 | 0.60 | 0.220 | 0.642 |
| Leptin (ng/mL) | 15.95 | 13.25 | 12.74 | 1.840 | 0.281 |
| IGF-1 (ng/mL) | 277.73 | 257.86 | 273.91 | 17.539 | 0.663 |

Abbreviations: VLDL = Very-low density lipoprotein; LDL = low-density lipoprotein; HDL = high density lipoprotein; SUN = serum urea nitrogen; IGF-1 = Insulin-like growth factor 1;

SEM = Standard error of means; * Post-weaning dry-to-rainy transition period before the induction protocol.
[a-b] Different letters declare significantly different between parities at $P < 0.05$.