

DOUGLAS TEIXEIRA SARAIVA

**EFFECT OF SUPPLEMENT LEVELS ON *CREEP-FEEDING* FOR SUCKLING
CALVES AND PREPARTUM NELLORE COWS ON PASTURE**

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*.

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Co-advisers: Luciana Navajas Rennó
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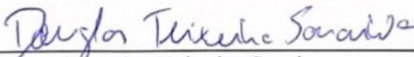
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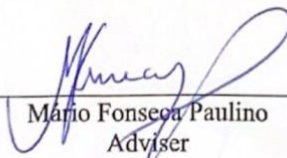
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*To my parents, Benjamim and Marilda, my
brother Autieres and my girlfriend Érica .*

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“No one will hit as hard as life. But it's not about how hard you can hit. It's about how hard you can be hit and keep moving forward. This is how victory is won.”

(Rocky Balboa)

BIOGRAPHY

Douglas Teixeira Saraiva, son of Benjamin Saraiva de Faria and Marilda Tereza Gomes Teixeira Faria, was born on May 9, 1992, in the town of Viçosa, Minas Gerais.

He started the undergrad in Animal Science at Federal University of Viçosa in 2010 and became a Bachelor of Science in Animal Science in 2015. At the same year she started the M.S. in agronomy. In July of 2017 he became a M.S. in Agronomy.

In 2019, he started his doctorate in Animal Science with specialization in ruminant nutrition and beef cattle production, submitting his thesis defense on February 28, 2023.

ABSTRACT

SARAIVA, Douglas Teixeira, D.Sc., Universidade Federal de Viçosa, February, 2023. **Effect of supplement levels on *creep-feeding* for suckling calves and prepartum Nellore cows on pasture.** Adviser: Mário Fonseca Paulino. Co-advisers: Luciana Navajas Rennó and Sebastião de Campos Valadares Filho.

In the first experiment, the study aimed to evaluate the effects of different levels of supplementation on the nutritional and productivity performance and metabolic parameters in beef calves fed in tropical grass pastures and the costs of this supplementation. Thirty-five Nellore male calves, with an average body weight (BW) of 115 ± 12 kg and an average age of 110 ± 10 days, and their dams (BW = 505 ± 25 kg, 8 years of age) were used. The supplementation levels were as follows: 0 = in the control group, calves received only a mineral mixture; one group received 5 g/kg BW of supplementation with 250 g of crude protein/kg of dry matter; and another group received 10 g/kg BW with 250 g of crude protein/kg of dry matter. The experimental design was completely randomized. The cow-calf pairs were randomly assigned to an experimental area of eight plots, and a group of four to five animals was considered one experimental unit. The intakes of dry matter (DM), organic matter (OM), crude protein (CP), and total digestible nutrients (TDN) ($P < 0.05$) were greater for calves that received a higher level of supplementation. However, supplementation did not affect the milk production and productive performance of the cows ($P > 0.05$). Supplementation increased the apparent digestibility of DM, OM, CP, and non-fiber carbohydrates (NFC), with digestibility increasing as inclusion of the supplement increased ($P < 0.05$). The calves receiving 10 g/kg BW supplementation showed a higher average daily gain (ADG) and thus a higher weight at weaning ($P < 0.05$) and higher concentrations of blood urea nitrogen (BUN) and insulin-like growth factor 1 (IGF-1). However, considering the good quality of the forage offered during the study period, an improvement in performance and nutritional characteristics was observed for the 10 g/kg BW treatment, however, without economic gain in the non-supplemented animals. In the second experiment, the objective was to evaluate the effects of different supplementation strategies for Nellore cows 60 days prepartum on performance, metabolic responses and influence on the initial body development of the offspring. We used 39 pluriparous, gestating male Nellore cows with an initial mean of 224 ± 2.6 days of pregnancy, 520 ± 15.2 kg body weight (BW) and 6.0 ± 0.07 (scale of 1 - 9) body condition score (BCS), according to completely randomized design, with four treatments and two repetitions. The treatments evaluated were: control receiving only

mineral mixture *ad libitum*, 2 g/kg BW, 4 g/kg BW and 6 g/kg BW of protein-energy supplement per cow per day for 60 days before calving. The supplement was formulated to contain similar amounts of crude protein, 300 g BW/kg in DM. Statistical evaluations were conducted using the PROC MIXED procedure of SAS (version 9.4), adopting $\alpha = 0.05$. Cows supplemented with 4 and 6 g/kg BW showed greater BW variation in the prepartum and less variation in the postpartum ($P < 0.05$). There was a higher BCS for supplemented animals ($P < 0.05$) at calving and at 90 days postpartum, with animals in the 4 and 6 g/kg BW treatment being higher than the control treatment ($P < 0.05$). There were no differences in calf birth weight and performance up to 90 days postpartum ($P > 0.05$). Animals receiving 4 and 6 g/kg BW had a shorter service period compared to the control treatment ($P < 0.05$), but there were no differences for overall pregnancy outcome ($P > 0.05$). Treatment x day interaction was observed for BUN which was higher in the prepartum for animals that received higher supplementation levels ($P < 0.05$), β HB, was higher in the prepartum for animals in the control treatment ($P < 0.05$), and for NEFA, concentrations were lower 21 days before calving and at 21 and 42 days after calving, for the treatments that received 4 and 6 g/kg BW ($P < 0.05$). Progesterone concentrations presented a positive linear effect with the increase of supplement supply ($P < 0.05$), being higher for animals that received 4 and 6 g/kg BW compared to animals in the control treatment ($P < 0.05$). In view of these results, Providing 4g/kg BW of protein-energy supplement to grazing Nellore cows 60 days prior to calving is recommended, which improves metabolic characteristics and performance in the prepartum and postpartum period and a lower negative energy balance in the postpartum period, resulting in a shorter service period.

Keywords: *Creep-feeding*. Cows. Nutrition. Physiology. NEFA. Progesterone.

RESUMO

SARAIVA, Douglas Teixeira, D.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Efeito de níveis de suplemento no *creep-feeding* para bezerros em lactação e vacas Nelore no pré-parto em pastejo.** Orientador: Mário Fonseca Paulino. Coorientadores: Luciana Navajas Rennó e Sebastião de Campos Valadares Filho.

O primeiro experimento, teve como objetivo avaliar os efeitos de diferentes níveis de suplementação sobre o desempenho nutricional e de produtividade e parâmetros metabólicos em bezerros de corte alimentados em pastagens de capim tropical e os custos desta suplementação. Foram utilizados 35 bezerros machos Nelore, com um peso corporal (PC) médio de 115 ± 12 kg e uma idade média de 110 ± 10 dias, e suas mães (PC = 505 ± 25 kg, 8 anos de idade). Os níveis de suplementação foram os seguintes: 0 = no grupo controle, os bezerros receberam apenas uma mistura mineral; um grupo recebeu 5 g/kg de PC de suplementação com 250 g de proteína bruta/kg de matéria seca; e outro grupo recebeu 10 g/kg de PC com 250 g de proteína bruta/kg de matéria seca. O delineamento experimental foi inteiramente casualizado. Os pares vaca-bezerro foram designados aleatoriamente para uma área experimental de oito piquetes, e um grupo de quatro a cinco animais foi considerado uma unidade experimental. A ingestão de matéria seca (MS), matéria orgânica (MO), PB e nutrientes totais digeríveis (NDT), foi maior para bezerros que receberam um nível maior de suplementação ($P < 0,05$). Entretanto, a suplementação não afetou a produção de leite e o desempenho produtivo das vacas ($P > 0,05$). A suplementação aumentou a digestibilidade aparente de MS, MO, PB e carboidratos não fibrosos (CNF), com a digestibilidade aumentando à medida que aumentava a inclusão do suplemento ($P < 0,05$). Os bezerros que receberam 10 g/kg de suplemento de PC apresentaram um maior ganho médio diário (GMD) e, portanto, um peso maior no desmame ($P < 0,05$) e maiores concentrações de nitrogênio uréico no sangue (NUS) e fator de crescimento 1 semelhante à insulina (IGF-1). Entretanto, mesmo considerando a boa qualidade da forragem oferecida durante o período de estudo, observou-se uma melhora no desempenho e nas características nutricionais para o tratamento de 10 g/kg de PC, sem ganho econômico nos animais não suplementados. No segundo experimento, o objetivo foi avaliar os efeitos de diferentes estratégias de suplementação para vacas Nelore 60 dias pré-parto no desempenho, respostas metabólicas e influência no desenvolvimento corporal inicial da prole. Foram utilizadas 39 vacas Nelore pluríparas e gestantes de fetos machos, com média inicial de $224 \pm 2,6$ dias de gestação, $520 \pm 15,2$ kg de peso corporal (PC) e $6,0 \pm 0,07$ (escala de 1 – 9) de escore de condição corporal (ECC),

segundo delineamento inteiramente casualizado, com quatro tratamentos e duas repetições. Os tratamentos avaliados foram: controle recebendo apenas mistura mineral *ad libitum*, 2 g/kg PC, 4 g/kg PC e 6 g/kg PC de suplemento proteico-energético por vaca ao dia durante 60 dias antes do parto. O suplemento foi formulado para conter quantidades semelhantes de proteína bruta, 300 g PB/kg na MS. As avaliações estatísticas foram conduzidas por intermédio do procedimento PROC MIXED do SAS (versão 9,4), adotando-se $\alpha = 0,05$. As vacas suplementadas com 4 e 6 g/kg PC apresentaram maior variação de PC no pré-parto e menor variação no pós-parto ($P < 0,05$). Houve um maior ECC para os animais suplementados ($P < 0,05$) no parto e aos 90 dias pós-parto, sendo que os animais do tratamento 4 e 6 g/kg PC foram superiores ao tratamento controle ($P < 0,05$). Não houve diferenças no peso ao nascimento dos bezerros e no desempenho até os 90 dias pós-parto ($P > 0,05$). Os animais que receberam 4 e 6 g/kg PC apresentaram um menor período de serviço em relação ao tratamento controle ($P < 0,05$), porém, não houve diferenças para o resultado de prenhez geral ($P > 0,05$). Foi observado interação tratamento x dia para BUN que foi maior no pré-parto para os animais que receberam maiores níveis de suplementação ($P < 0,05$), o β HB, foi superior no pré-parto para os animais do tratamento controle ($P < 0,05$), e para o NEFA, as concentrações foram menores 21 dias antes do parto e aos 21 e 42 dias após o parto, para os tratamentos que receberam 4 e 6 g/kg PC ($P < 0,05$). As concentrações de progesterona apresentaram um efeito linear positivo com o aumento da oferta de suplemento ($P < 0,05$), sendo superior para os animais que receberam 4 e 6 g/kg PC em comparação aos animais do tratamento controle ($P < 0,05$). Recomenda-se fornecer 4g/kg de PV de suplemento protéico-energético às vacas Nellore em pastagem 60 dias antes do parto, o que melhora as características metabólicas e o desempenho no período de preparação e pós-parto e um balanço energético negativo mais baixo no período pós-parto, resultando em um menor período.

Palavras-chave: *Creep-feeding*. Vacas. Nutrição. Fisiologia. NEFA. Progesterona.

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1. INTRODUCTION

In an ever-changing world, with increasing global demand for food, providing food for the entire human population has become increasingly challenging (Ruggieri et al., 2020; Tedeschi et al., 2015). By 2050, the global population is estimated to be 12 billion, which will be accompanied by decreasing global poverty and increasing food consumption in developing and underdeveloped countries. Highly populated Asian countries, such as China and India, will have a substantial increase in the consumption of animal protein, especially beef (Mcauliffe et al., 2018).

Population growth and pressures on the availability of productive land for cattle ranching underpin the need for improvements in the productivity and efficiency of beef production (Capper & Bauman, 2013) and in a more sustainable manner. This enormous challenge, however, must be overcome in increasingly restricted scenarios in terms of resource availability, including water and land available for agricultural activities. Therefore, there is a search to produce more per unit of resource used, i.e., more efficiently (Medeiros et al., 2013). On beef farms that are more advanced in development, these objectives are supported by continuous improvements in genetics, nutrition, and management practices. These practices will need to increase reproductive and growth efficiency, economic, animal welfare, and environmental outcomes (Greenwood, 2021).

Brazil has had the highest growth rate in beef production of any major meat producing country globally since 2008 and is the world's largest beef exporter (ABIEC, 2021; FAOSTAT, 2020). Featuring about 196.4 million head of cattle in 2021, predominantly *Bos indicus* cattle. Beef production of 11 million tons contributed about 9% to the national gross domestic product in 2018, when over 44 million head were slaughtered (ABIEC, 2021). However, productivity in Brazil has been gaining on its main international competitors through improved genetics and meat management and increasing the number of cattle in feedlots (MLA, 2020). China was Brazil's largest export market by value in 2018, followed by Hong Kong, the European Union, Egypt, and Chile (Greenwood, 2021).

There are about 162 million hectares of pastureland, which is equivalent to 19% of Brazil's 852 million hectares, and another 71 million hectares of perennial, semi-perennial and annual farmland (ABIEC, 2021). Considering the period from 1990 to 2018 Brazilian meat productivity increased by about 176%. In this period, there was an increase in productivity per ha from 24.5 to 67.5 kg of beef, and an increase from 4.6 to 11.0 million tons of total beef production. The area of pasture in Brazil was reported to have decreased from 192 to 162

million hectares between 1990 and 2018 due to reduced deforestation and area with pasture according to ABIEC, (2021).

Tropical pastures are the main source of nutrients for beef cattle in Brazil, standing out from other means of feeding by the low cost of production and high practicality (Paulino et al., 2008; Valle et al., 2020), and with this, meat production can be practiced in a sustainable way (Greenwood, 2021).

However, throughout the year, pastures are subject to variations in the availability and quality of their constituents (Valle et al., 2020). However, grazed forage should be understood as a basal nutritional resource of high complexity, since its ability to provide substrates for animal production varies qualitatively and quantitatively throughout the year depending mainly on the influence of climatic variables (Detmann et al., 2010). It can present multiple nutritional deficiencies; this imbalance is not only observed during the dry season, but also in the rainy season (Valente et al., 2013). Tropical pastures rarely constitute a balanced diet in the sense that their organic and inorganic constituents are present in the concentrations and proportions that meet the needs of the animals. Therefore, cattle generally suffer multiple deficiencies of protein, energy, minerals, and vitamins (Paulino et al., 2014). With this, the animal performance may be lower than the determined genetic potential and may not meet the production objectives (Valle et al., 2020).

Technical intervention has always been focused on the final fattening process, of greater visibility and tangible and immediate result (Paulino et al., 2014). However, the success of this stage depends on the quality of the animals produced in the rearing stage. However, until the production of a calf, beef breeder cows demand a large investment (Lopes, et al., 2017). Despite the prominent position, the Brazilian beef cattle industry still has low reproductive rates, such as average age at first calving close to 40 months, high calving interval and a calf production rate close to 68% (Baruselli et al., 2017).

Nutritional status at parturition is the main factor influencing the duration between parturition and the next conception (Baruselli et al., 2004; Mulliniks et al., 2013). Inadequate energy supply in late pregnancy impairs reproduction, even when it is sufficiently offered during lactation. However, for the breeding process to be considered efficient, cows need to produce a calf every 12-13 months (Torres Jr. et al., 2009). which can directly impact future calf performance, with calves born earlier in the calving season having higher body weight at weaning (Funston et al., 2012).

On the other hand, in addition to the reproductive efficiency discussed above, intensive beef cattle farming is characterized by the mating of heifers at 14-16 months of age,

and the slaughter of males between 11 and 16 months of age. Thus, continuous weight gains from birth to weaning are fundamental to the success of the system (Paulino et al., 2012). (Paulino et al., 2003). Weight gain at calf weaning can improve the economics of the cow-calf operation. Furthermore, by increasing the performance of lactating calves, days to slaughter can be reduced (Carvalho et al., 2019).

In this context, supplementation programs are tools for the provision of supplementary resources aimed at the reduction or elimination of nutritional and/or metabolic barriers and the achievement of animal production goals (Detmann et al., 2014). In summary, the supplementation of grazing cattle allows insertion of an additional source of nutrients, reflecting changes in forage intake, availability of dietary energy and, consequently, animal performance (Paulino et al., 2010).

In the tropics, beef cows typically spend most of their pregnancy period during the dry season, which is characterized by low forage yield and quality. Therefore, supplementation during the dry season can be an effective measure to improve the body condition of animals in the tropics (Paulino et al., 2010; Detmann et al., 2014), as this is one of the most important factors in determining the extent of postpartum anestrus (Ayres et al., 2014).

On the other hand, excess nutrients in the prepartum period has also been associated with adverse effects on reproductive efficiency (Santos et al., 2008; Sartori et al., 2010; Wiltbank et al., 2006). However, there is little information with *Bos indicus* and many inconsistencies in the literature regarding the effects of prepartum supplementation on the productive and reproductive performance of beef cows during this period.

Changes in metabolite concentrations during peripartum can be interpreted as metabolites that link nutrition and physiology (Payne, 1987), and can help to accurately indicate the effects of supplementation on animal metabolism (Lana Ferreira et al., 2020).

In addition, since calves during lactation have a rapid growth rate (Owens et al., 1993), leveraging these gains is of paramount importance. However, under tropical conditions, even with more productive cows, by the third month of age, milk production is insufficient to meet the needs of calves to support potential growth (Costa e Silva et al., 2015). Thus, for intensive cattle production systems, which require greater nutritional input, supplementation of lactating animals under the creep-feeding system (Paulino, 2008) is envisioned. This refers to the provision of additional food for animals in the lactation phase, in a place whose access is restricted to calves (Paulino et al. 2012).

However, there are questions about the economic viability of supplementation in the various phases of production, about the appropriate strategies to be used in each phase, and even about the productive and metabolic response that can be achieved.

Therefore, studies are needed to evaluate the responses of beef cattle, *Bos indicus*, of different categories subjected to different supplementation plans while grazing. In this context, studies were conducted with the objective of:

- 1- To evaluate the effect of different levels of supplementation on nutritional performance, production, metabolic profile for suckling Nelore calves on tropical pastures.
- 2- To evaluate the effects of different supplementation strategies for Nelore cows 60 days prepartum on performance, metabolic responses, and influence on early body development of offspring.

2. REFERENCES

- Aeberhard.K; Bruckmaier, R.M; Blum, J.W. Metabolic, enzymatic and endocrine status in high yielding dairy cows – part 2. *Journal of Veterinary Medicine Series*, v. 8, p.111-127, 2001.
- Astessiano, A.L; Pérez-Clariget; Espasandí, A.C. et al. Metabolic, productive and reproductive responses to postpartum short-term supplementation in primiparous beef cows. *Revista Brasileira de Zootecnia*, v. 42, p. 246-253, 2013.
- Associação Brasileira das Indústrias Exportadoras de Carne – ABIEC. 2021. Beef Report – Perfil da Pecuária no Brasil. <https://www.abiec.com.br/publicacoes/beef-report-2021/>. Accessed 15 Jan 2023.
- Ayres, H.; Ferreira, R. M.; Torres-Júnior, J. R. S.; Demétrio et al. Inferences of body energy reserves on conception rate of suckled Zebu beef cows subjected to timed artificial insemination followed by natural mating. *Theriogenology*, v. 82, n. 4, p.529-536, 2014.
- Baruselli, P. S; Reis, E.L; Marques, L.F; Nasser, G. A. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Animal Reproduction Science*, v. 82–83, p. 479–486, jul. 2004.
- Baruselli, P. S; Ferreira, R.M; Colli, M. H. A. et al. Timed artificial insemination: Current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. *Animal Reproduction*, v. 14, n. 3, p. 558–571, 2017.
- Capper, J. L.; Bauman, D. E. The role of productivity in improving the environmental sustainability of ruminant production systems. *Annual Review of Animal Biosciences*,

- v. 1, p. 469–489, 2013.
- Carvalho, V.V., Paulino, M.F., Detmann, E., Filho, S.C.V., Lopes, S.A., Rennó, L.N., Sampaio, C.B., Silva, A.G., 2019. A meta-analysis of the effects of creep-feeding supplementation on performance and nutritional characteristics by beef calves grazing on tropical pastures. *Livestock Science* 227, 175-182.
- Costa e Silva, L.F., Engle, T.E., de Valadares Filho, S., Rotta, P.P., Villadiego, F.A.C., Silva, F.A.S., Martins, E.C., Silva, L.H.R., Paulino, M.F., 2015. Nellore cows and their calves during the lactation period: performance, intake, milk composition, and total apparent digestibility. *Trop Anim Health Prod* 47, 735–741.
- Detmann, E., Paulino, M.F., Valadares filho, S.C. 2010. Otimização do uso de recursos forrageiros basais. In: *Anais do 7o Simpósio de Produção de Gado de Corte*, Viçosa. p.191-240.
- Detmann, E., Paulino, M.F., Valadares Filho, S.D.C., Huhtanen, P., 2014. Nutritional aspects applied to grazing cattle in the tropics: a review based on Brazilian results. *Sem. Ci. Agr.* 35, 2829. <https://doi.org/10.5433/1679-0359.2014v35n4Suplp2829>
- FAOSTAT, 2020. Food and agriculture data. Retrieved on 15 January 2023 from <https://www.fao.org/faostat/en/#home>.
- Funston, R.N; Musgrave. J.A; Meyer, T.L; Larson, D.M. Effect of calving distribution on beef cattle progeny performance. *J Anim Sci.* v.90. p.5118–21, 2012.
- Greenwood, P. L. Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. *Animal*, p. 100295, jul. 2021.
- Lana Ferreira, M. F; Rennó, L. N.; Detmann, E. et al. Performance, metabolic and hormonal responses of grazing Nellore cows to an energy-protein supplementation during the pre-partum phase. *BMC Veterinary Research*, v. 16, n.1, p.1-13. 2020.
- 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., 2017. Evaluation of supplementation plans for suckling beef calves managed on tropical pasture. *SCA* 38, 1027. <https://doi.org/10.5433/1679-0359.2017v38n2p1027>
- McAuliffe, G. A., Takahashi, T., Orr, R. J., Harris, P., & Lee, M. R. F. (2018). Data to calculate emissions intensity for individual beef cattle reared on pasture-based production systems. *Data in brief*, 17, 570-574, 2018.
- Medeiros, S. R. de; Gomes, R. da C.; Nascimento, M. L. do; Albertini, T. Z.; Souza, A. R. D. L. dos; Reis, S. F. dos; Paulino, P. V. R. P.; Lanna, D. P. Eficiência nutricional: chave

- para a produção sustentável de carne bovina. Embrapa Gado de Corte. p. 61-75, 2013.
- MLA. Global snapshot - Beef. Meat & Livestock Australia, North Sydney, Australia. 2020.
- Mulliniks, J. T; Kemp, M. E; Endecott, R. L. et al. Does β -hydroxybutyrate concentration influence conception date in young postpartum range beef cows. *Journal of Animal Science*, v. 91, n. , p.2902-2909, 2013.
- Paulino, M. F; Detmann, E; Valente E. E. L. 2008. Nutrição de bovinos em pastejo. In: *Simpósio Sobre Manejo Estratégico da Pastagem*. Viçosa. p. 131–169.
- Paulino, M. F.; Detmann, E.; Silva, A. G. et al. 2014. Bovinocultura Otimizada In: *Simpósio de produção de gado de corte*, 9, Viçosa, MG. Anais. Viçosa, MG: Simcorte. p. 139-164.
- Paulino, M. F.; Detmann, E; Silva, A. G. et al. 2014. Bovinocultura Otimizada In: *Simpósio de produção de gado de corte*, 9, Viçosa, MG. Anais. Viçosa, MG: Simcorte. p. 139-164.
- Payne, J.M; Payne, S. The metabolic profile test. Oxford: Oxford University, in press; 1987.
- Owens, F.N., Dubeski, P., Hanson, C.F., 1993. Factors that alter the growth and development of ruminants. *J. Anim. Sci.* 71 (11), 3138–3150.
- Ruggieri, A. C., Cardoso, A. D. S., Ongaratto, F., Casagrande, D. R., Barbero, R. P., Brito, L. D. F. Grazing Intensity impacts on herbage mass, sward structure, greenhouse gas emissions, and animal performance: analysis of brachiaria Pastureland. *Agronomy*, 10(11), 1750, 2020.
- Santos, J. E. P; Cerri, R. L. A; Sartori, R. Nutritional management of the donor cow. *Theriogenology*, v. 69, n.1, p.88-97, 2008.
- Sartori, R.; Guardieiro, M. M. Fatores nutricionais associados à reprodução da fêmea bovina. *Revista Brasileira de Zootecnia*, v. 39, n. SUPPL. 1, p. 422–432, 2010.
- Tedeschi, L. O., Muir, J. P., Riley, D. G., & Fox, D. G. (2015). The role of ruminant animals in sustainable livestock intensification programs. *International Journal of Sustainable Development & World Ecology*, 22(5), 452-465, 2015.
- Torres Junior, J. R. S.; Mello, W. O.; Elias, A. K. S.; Rodrigues, L. S.; Pentead, L.; Baruselli, P. S. Considerações técnicas e econômicas sobre reprodução assistida em gado de corte. *Revista Brasileira de Reprodução Animal*, v. 33, p.53-58, 2009.
- Valente, E. E. L.; Paulino, M.F.; Barros, L.V.; Almeida, D.M.; Martins, L.S.; Cabral, C.H.A. Effect of calves" supplementation on performance, nutritional and behavioral characteristics of their dams. *Tropical Animal Health and Production*, v.45, p.487-495, 2013.

- Valle, F. R., Fontes, C. A., Fernandes, A. M., Oliveira, T. S., Processi, E. F., & Silva, R. S. (2020). Performance, digestibility, microbial protein synthesis, and body composition of Brangus x Zebu steers on tropical pasture receiving supplementation. *Tropical Animal Health and Production*, 52(5), 2491-2498, 2020.
- Wiltbank, M; Lopez, H; Sartori, R. et al. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*, v. 65, n.1, p. 17-29, 2006.

MANUSCRIPT 1: PERFORMANCE, NUTRITIONAL AND METABOLIC CHARACTERISTICS OF SUPPLEMENTED SUCKLING BEEF CALVES ON PASTURE

ABSTRACT

This study aimed to evaluate the effects of different levels of supplementation on the nutritional and productivity performance and metabolic parameters in beef calves fed in tropical grass pastures and the costs of this supplementation. Thirty-five Nellore male calves, with an average body weight of 115 ± 12 kg and an average age of 110 ± 10 days, and their dams (BW = 505 ± 25 kg, 8 years of age) were used. The supplementation levels were as follows: 0 = in the control group, calves received only a mineral mixture; one group received 5 g/kg BW of supplementation with 250 g of crude protein/kg of dry matter; and another group received 10 g/kg BW with 250 g of crude protein/kg of dry matter. The experimental design was completely randomized. The cow-calf pairs were randomly assigned to an experimental area of eight plots, and a group of four to five animals was considered one experimental unit. The intakes of dry matter (DM), organic matter (OM), crude protein (CP), and total digestible nutrients (TDN) ($P < 0.05$) were greater for calves that received a higher level of supplementation. However, supplementation did not affect the milk production and productive performance of the cows ($P > 0.05$). Supplementation increased the apparent digestibility of DM, OM, CP, and non-fiber carbohydrates (NFC), with digestibility increasing as inclusion of the supplement increased ($P < 0.05$). The calves receiving 10 g/kg BW supplementation presented a higher average daily gain (ADG) and thus a higher weight at weaning ($P < 0.05$) and higher concentrations of blood urea nitrogen (BUN) and insulin-like growth factor 1 (IGF-1), and they had greater thickness of subcutaneous fat in the rib ($P < 0.05$). Given the results obtained in this study, under conditions of good forage quality, supplementation containing 250 g CP/kg DM at 10 g/kg BW is recommended for suckling Nellore calves on tropical pastures. Supplementation increases dry matter intake, improves metabolic characteristics, body composition and performance of calves.

Keywords: Creep-feeding. Nellore. Suckling. Supplementation. IGF-1

Abbreviations: ADG, average daily gain; BUN, urea nitrogen in the blood; BW, body weight; CP, crude protein; DM, dry matter; IGF-1, insulin-like growth factor; NFC, non-fiber carbohydrates; OM, organic matter; TDN, total digestive nutrients

1. INTRODUCTION

An increase in the weight of calves at weaning might improve the profitability of a cow-calf operation. Additionally, an increase in the performance of suckling calves can lead to a reduction in the days until slaughter (Carvalho et al., 2019). In tropical climates, a cow's milk production is insufficient to meet calves' potential growth by their third month, even with more productive cows (Costa e Silva et al., 2015). Consequently, calves depend significantly on pasture to supplement their diets. However, this occurs during the transitional rainy-dry period, when the forage available for pasture in most production systems in Brazil drops in quality and quantity (Costa e Silva et al., 2016). Therefore, creep-feeding has arisen as a way to provide supplementation to suckling beef calves in intensive bovine production systems which require greater nutritional support (Paulino, 2008).

The young calves' quick growth rate (Owens et al., 1993) suggests that supplementation at this phase could be a very effective approach. However, the cost-effectiveness of a supplementation program depends on the cost of supplement, calf prices, and, eventually, the supplemental feed efficiency (Aguilar et al., 2015). In addition to calf genetics, feed efficiency could be related to the amount and composition of the supplement. A few studies have evaluated the levels of supplementation in suckling calves (Barros et al., 2015, 2015; Cremin et al., 1991), and some results have shown that high levels of supplementation have decreased fiber digestion (Cremin et al., 1991; Lopes et al., 2017) and negatively affected calf performance (Lopes et al., 2017). These results, however, may vary depending on the season and the nutritive value and amount of forage (Adams et al., 2000).

Nonetheless, the effects of supplementation have not been thoroughly explored, and little is known about the effects of high levels of supplementation on metabolic responses and body composition in suckling calves. The hypothesis is that higher levels of supplementation improve performance, metabolic responses, and body composition in Nellore beef calves in the suckling phase. This study aimed to evaluate the effects of different levels of supplementation on nutritional and productive performance and metabolic parameters in suckling Nellore calves under tropical pastures.

2. MATERIALS AND METHODS

2.1. Animals, experimental design, and treatments

All procedures involving the use of animals were approved by the Ethics Committee for the Use of Research Animals of Federal University of Viçosa under a protocol (CEUAP-

UFV 143/19). The experiment was conducted at the Unidade de Ensino, Pesquisa e Extensão em Gado de Corte (UEPE-GC), Universidade Federal de Viçosa, MG, Brazil (20° 45 ' S 42 ° 52 ' W) between February and July 2020. The average temperature and precipitation were 22.6° C and 1165 mm respectively (Fig. 1).

Thirty-five male Nellore calves with an average initial body weight (IBW) of 115 ± 12 kg and an average age of 110 ± 10 and their respective dams (IBW = 505 ± 25 kg, 8 years of age) were used in the experiment. The treatments were as follows: 0 g/kg BW (control group), without supplementation concentrate; 5 g/kg BW supplementation; and 10 g/kg BW supplementation. The supplement was formulated to contain a similar amount of CP (250 g CP/kg DM; Tables 1 and 2). All the animals received a mineral mixture *ad libitum*, containing dicalcium phosphate (500 g/kg), sodium chloride (472 g/kg), zinc sulfate (15 g/kg), copper sulfate (7 g/kg), manganese sulfate (5 g/kg), cobalt sulfate (0.5 g/kg), sodium selenite (0.06 g/kg), and potassium iodate (0.5 g/kg).

Cow-calf pairs were randomly assigned to an experimental area of eight plots, where each group of four to five animals was considered an experimental unit. Each group was placed on a 7.5-ha plot of pasture consisting of *Urochloa decumbens*. There were private feeders for the calves (*creep-feeding*). Cows received *ad libitum* mineral mixture in a separate feeder. Water was offered *ad libitum* during the experiment. Animals were submitted to 14 days of diet and management adaptation before the beginning of the experimental period, when all the calves received 5 g/kg BW of supplementation. The experimental period lasted 140 days; at the end, the calves were weaned at approximately 8 months of age.

Calves were fed daily at 11 am, and adjustments to the amount of supplement were made every 30 days, after weighing the animals, always at the same time (6 am). To minimize the effects of the plot on treatments, the animals were rotated between plots every seven days.

2.2. Experimental procedures and sampling

Calves were weighed to track BW variation, after 14 hours of fasting on solids and liquid at the beginning and end of the experimental period; then, the average daily gain (ADG) was calculated, which is the difference in weight gain divided by days of the experimental period. For cows, the weighing was performed without fasting, and body condition score (BCS) assessments on a scale of 1 to 9 were performed by three trained people at the beginning and end of the experiment, as recommended by the NRC (2016).

2.3. Forage samples

Forage samples were collected every 30 days by the hand-plugged method to evaluate the forage selected by the animal. To quantify the potential digestive dry matter (pdDM) available, forage samples were randomly selected and cut at approximately 5 cm above the ground using a metal square (0.5×0.5 m) in each plot. All the samples were weighed, dried (55°C), and ground in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, EUA) into 2 and 1 mm, and stored for further analysis.

2.4. Intake, digestibility, and nitrogen balance trial

To evaluate the nutritional characteristics of the calves' diet, 70 days after the beginning of the experiment (at 190 days of age), a nine-day digestibility trial was performed using the three markers method. The first six days were used to establish markers flow in the gastrointestinal tract of the calves, and the last four for collection of fecal samples at 6 pm, 2 pm, 10 am, and 6 am. Chromium oxide (Cr_2O_3) was used to estimate fecal excretion, an external marker (Detmann et al., 2001). Each calf received 10g/day, packed in a paper cartridge, and it was directly introduced into the esophagus via a metal tube at 11 am during the digestibility trial. Titanium dioxide (TiO_2) was included directly in the supplement (Titgemeyer et al., 2001), 10g/kg of offered supplement to estimate the supplement individual intake. On the fifth day of the digestibility period, a manual grazing simulation was performed to estimate voluntary pasture intake, where indigestible neutral detergent fiber (iNDF) was used as an internal markers to estimate forage dry matter (DM) intake (Detmann et al., 2001). The fecal samples were identified, dried in a forced ventilation oven (55°C), ground in a knife mill to 2 and 1 mm, proportionally subsampled into a composite sample per animal, and processed as described above.

After the nine-day intake and digestibility trial, samples of urine "spot" were collected, in spontaneous urination, to evaluate the production of microbial protein. Collections were performed four hours before and four hours after supplementation (Silva Júnior et al., 2021). After collection, the urine samples were proportionally subsampled into a composite sample per animal and then separated into a concentrated sample (10 mL); another sample was diluted in 40 mL of H_2SO_4 (0.036 N) and frozen (-20°C) for further analysis.

2.5. Milk sample

To estimate the milk yield, cows were milked on the 160th and 200th days of lactation. To remove all the milk from the udder, calves were separated from their dams at 3 pm, and then at 5:45 pm they were reunited so the calves could suckle the milk. At 6 pm, the calves

were separated again until the following morning, remaining housed in pens with access to water. Cows were milked at 5 am immediately after injection of 2 mL of oxytocin (10 UI/mL, Ocitopec®, Biovet, Sao Paulo, Brazil) in the mammary vein. The milk was weighed, and 30 mL was separated from each cow to evaluate the milk composition. The order and time that each cow was milked were recorded, and, to quantify the milk yield in 24h, the calves were kept apart from their dams until the next milking at 6 pm.

2.6. Blood sample

To evaluate the metabolic profile, blood was collected on days 150, 180, 210, and 240, corresponding to the average age of the calves. Blood samples were collected at 7 am, before feeding. The blood was collected by jugular venipuncture using vacuum tubes with separator gel (SST II Advance®, BD Vacutainer, Sao Paulo, Brazil) to quantify concentrations of urea, total protein, albumin, triglycerides, and insulin-like growth factor 1 (IGF-1). Additionally, glucose plasmatic concentration was quantified using EDTA and sodium fluoride tubes (BD Vacutainer® Fluorinated/EDTA, Sao Paulo, Brazil). All samples were centrifuged at 3600xg for 20 minutes, and the serum and plasma were immediately frozen at -20°C.

2.7. Body composition

On days 150, 180, 210, and 240 the average age of the calves, ultrasound measurements of the rib-eye area (REA), rump depth, rump fat thickness, and 12th rib fat thickness were taken using an Aloka (SSD 500V®, Aloka, Ltda., Tokyo, Japan) ultrasound equipped with the linear probe with 18 cm of length; the images were analyzed by the BioSoft Toolbox® II for beef (Biotronics Inc., Ames, Iowa, USA) program.

2.8. Chemical analysis

Forage, feces, and supplement ingredient samples previously ground to 1 mm were analyzed following the standard analytical procedures of the Instituto Nacional de Ciência e Tecnologia em Ciência Animal (INCT-CA; Detmann et al., 2021), for DM (INCT-CA method G-003/1), crude protein (CP; INCT-CA method N-001/1), mineral matter (MM; INCT-CA method M-001/1), ether extract (EE; INCT-CA method G-004/1), and neutral detergent fiber (aNDF; INCT-CA method F-002/1), using alpha thermostable amylase without sodium sulfite and corrected for ash residue (CIDN; INCT-CA method M-002/1) and protein (PIDN; INCT-CA method N-004/1). To quantify indigestible neutral detergent fiber (iNDF; INCT-CA method F-009/1), an *in situ* incubation procedure was performed using a non-woven textile bag (100 g/m²) for 288 h of samples ground to 2mm. In addition, the feces

samples were evaluated for chromium (INCT-CA method M-005/1) and titanium dioxide (INCT-CA method M-007/1) content.

Uric acid, creatinine, and urea were analyzed using Bioclin® (K0139, K067 e K056, Belo Horizonte, Brazil) kits, determined by an automated biochemical analyzer (BS200E Mindray, Shenzhen, China). Allantoin was analyzed using the colorimetric method (Chen e Gomes, 1992).

For blood samples, Bioclin® (Belo Horizonte, Brazil) kits were used to quantify urea (K056), total protein (K031), albumin (K040), and triglycerides (K117) in the serum; the plasma glucose concentration (K082) was quantified using automatic equipment for biochemistry, model BS200E (Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China). The insulin-like growth factor 1 (IGF-1) concentrations were quantified using DiaSorin® in an automated chemiluminescence analyzer (Liaison®, Italy) kit.

Milk protein, milk protein, lactose, and solids were analyzed using an infrared spectrophotometer (Foss MilkoScan FT120, Sao Paulo, Brazil).

2.9. Calculations

The pdDM in the forage samples was obtained following Paulino et al. (2008):

$$pdDM = 0.98 * (100 - NDF) + (NDF - iNDF)$$

where 0.98 = true digestibility of cell content; NDF = neutral detergent fiber (%); iNDF = indigestible neutral detergent fiber (%).

Fecal excretion (FE, kg/day) was estimated by the ratio of the amount of chromium ingested (g) and its concentration in the feces (CFc, g/kg).

$$FE = (Cr/CFc) * 100$$

The individual DM intake of the supplement was estimated by the ratio of TiO₂ excretion in feces and the markers concentration in the supplement, based on the following equation:

$$DMS = (FETi)/(CTiO_2S)$$

where DMS = dry matter intake of supplement (kg/day); FETi = fecal excretion of titanium (g/day); and CTiO₂S = concentration of TiO₂ in the supplement provided to the group (g/kg).

The voluntary intake of forage dry matter (DMF) was estimated following Detmann et al. (2001), using iNDF as an internal markers, from the following equation:

$$DMF = [(FE * iNDF_f) - (DMS * iNDF_s)] / iNDF_{fo}$$

where FE = fecal excretion (kg/d); iNDF_f = concentration of iNDF in the feces (kg/kg); DMS = dry matter intake of supplement (kg/d); iNDF_s = concentration of iNDF in the supplement (kg/kg); and iNDF_{fo} = concentration of iNDF in the forage (kg/kg).

Quantification of non-fibrous carbohydrates (NFC) was performed following Detmann & Valadares Filho (2010):

$$NFC = 100 - (\%CP + \%apNDF + \%EE + \%MM)$$

With the nutrient digestibility coefficients, the intake of total digestible nutrients (TDN) was estimated using the following equation:

$$TDN = dCP + dapNDF + (dEE * 2.25) + dNFC$$

where dCP = digestible crude protein; dapNDF = digestible neutral detergent fiber corrected for ash and protein, dEE = digestible ether extract; and dNFC = digestible non-fibrous carbohydrates.

The excretion of urinary purine derivatives (allantoin and uric acid) was used to estimate microbial protein synthesis. The daily urine volume was estimated using the ratio between daily creatinine excretion (CE) and its concentration in urine. Daily excretion was estimated according to Valadares Filho et al. (2016).

$$CE (mg/day) = 37.88 * SBW^{0.9316}$$

where SBW = shrunk body weight (kg).

The total excretion of purine derivatives was calculated by summing the amounts of allantoin and uric acid excreted in the urine, using the equation proposed by Barbosa et al. (2011):

$$Y = (X - 0.30 * BW^{0.75}) / 0.80$$

where Y = absorbed purine (mmol/d); X = excretion of purine derivatives (mmol/d); 0.30 = endogenous excretion of purine derivatives in urine (mmol); BW^{0.75} = metabolic weight; and 0.80 = recovery of purines absorbed as purine derivatives in urine (mmol/mmol).

Rumen microbial nitrogen synthesis was calculated as a function of absorbed purines using the equation proposed by Barbosa et al. (2011):

$$Z = (70 * Y) / (0.93 * 0.137 * 1000)$$

where Z = ruminal microbial nitrogen synthesis (g/d); Y = purines absorbed; 70 = N content in purines (mg/mol); 0.93 = true microbial purine digestibility; and 0.137 = ratio of purine N to microbial N.

Microbial protein synthesis (MPS) was found by multiplying the value found for microbial nitrogen synthesis by 6.25. Rumen degradable protein (RDP) was adopted as equivalent to microbial protein synthesis (Valadares Filho et al., 2016), and rumen

undegradable protein (RUP) was estimated by the difference between the intake of CP and RDP. With the RDP and RUP values, the metabolizable protein (MP) was estimated using the following equation:

$$MP = (RDP \times 0.64) + (RUP \times 0.80)$$

where MP = metabolizable protein; RDP = rumen degradable protein; and RUP = rumen undegradable protein.

Microbial efficiency was obtained by the ratio of microbial nitrogen synthesis, expressed in grams, to the amount of organic matter digested (DOM), expressed in kilograms.

Blood urea nitrogen (BUN) concentrations were estimated as 46.67% of total serum urea, and globulin concentrations were calculated as the difference between total protein and albumin.

2.10. Statistical Analysis

The experiment was conducted and analyzed in an entirely randomized experimental design with double error structure. The results were analyzed adopting the initial body weight as covariate. The analyses of variance (ANOVA) for the variables studied were performed according to the following mathematical model:

$$Y_{ijk} = \mu + T_i + e_{(ij)} + \varepsilon_{(ij)k}$$

where Y_{ijk} = observation taken on individual k in paddock j subjected to treatment i ; μ = overall mean; T_i = fixed effect of treatment; $e_{(ij)}$ = random, unobservable error associated with each paddock j subjected to treatment i , NID assumption $(0, \sigma_e^2)$; and $\varepsilon_{(ij)k}$ = random, unobservable error associated with each observation k allocated to paddock j and subjected to treatment i , NID assumption $(0, \sigma^2\varepsilon)$.

Carcass and blood metabolite measurements were analyzed using the repeated measures procedure, for which the day of collection was considered the repeated variable. The most appropriate covariance structure was chosen based on the lowest value of the corrected Akaike information criterion. Partial budgeting was analyzed using descriptive statistics. For all statistical procedures, the PROC MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) was used, adopting $\alpha=0.05$ as the critical level for the probability of occurrence of type I error.

3. RESULTS

3.1. Forage samples and nutritional characteristics

The average availability of DM and pdDM during the experiment was 4087 and 2970 kg/ha, respectively. The forage samples collected by the hand-plucked method showed an average content of 106.7 g/kg DM of CP (Table 2).

The intake of DM, OM, CP, TDN, NFC, digestible organic matter (DOM), iNDF, and the relation CP:DOM was higher in the supplemented animals than in the non-supplemented animals ($P<0.05$; Table 3), increasing as the level of the supplement increased.

For dry matter forage intake (DMF; kg/day; Table 3), there were no differences between treatments ($P>0.05$). In contrast, when evaluating DMF, iNDF, and NDF in g/kg BW, the calves supplemented with 10 g/kg BW showed a lower forage intake than the other two treatments, which did not differ from one another ($P<0.05$; Table 3). There was no treatment effect on milk production and composition ($P>0.05$; Table 4).

Supplementation increased the apparent digestibility of DM, OM, CP, NFC, and DOM contents (g/kg DM⁻¹); except for the CP content, the increase was greater as the supplement increased ($P<0.05$; Table 5).

The increase in supplement intake changed the profile of the protein, showing an increase in the intake of RUP ($P<0.007$; Table 6) and MP ($P<0.001$). The increase in N intake ($P<0.001$) increased the retained N ($P<0.08$) only for the 10 g/kg BW treatment (Table 5), despite higher values of N excreted in feces ($P=0.018$) and urine ($P=0.003$). The same behavior was observed for microbial protein synthesis, which did not change the efficiency of the use of microbial nitrogen compounds (MNS; $P=0.254$) or the microbial efficiency (MPS/MOD; $P=0.329$).

3.2. Performance

The cows' final BW (kg) and BCS were not influenced by the treatments ($P>0.05$; Table 7). Animals in the 10 g/kg BW treatment group showed higher ADG and, consequently, greater weight at weaning ($P<0.05$; Table 7). Meanwhile, the animals in the control group and in the 5 g/kg BW treatment group showed no differences for these characteristics ($P>0.05$; Table 7).

An interaction between treatment and day ($P<0.05$) was observed for carcass characteristics and for evaluations of rib-eye area and 12th-rib fat thickness (Table 7; Fig. 2A and 2C). For the evaluations of rump depth and rump fat thickness (P8), a difference between treatments was observed; those evaluations were greater for the animals in the 10 g/kg BW

supplementation group and smaller for the control group ($P < 0.05$; Table 7; Fig. 2B and 2D). For all carcass characteristics, there was a day effect ($P < 0.05$; Table 7), increasing with the age of the calves (Fig. 2A, 2B, 2C, and 2D).

3.3. Metabolic characteristics

For blood metabolites, no interactions were observed between treatment and the day of the collection ($P > 0.05$; Table 7). The concentration of blood urea nitrogen (BUN) was higher in supplemented animals than in the non-supplemented animals ($P < 0.05$; Table 8), increasing from day 150 to 180 and then stabilizing for the supplemented treatments. By contrast, the non-supplemented animals showed a decrease in BUN after 210 days ($P < 0.05$; Table 8; Fig. 3A). IGF-1 concentrations were greater for animals in the 10 g/kg BW treatment group compared to the other two groups, which did not differ from one another ($P < 0.05$; Table 8); IGF-1 concentrations increased with the increase in the animals' age for all treatments ($P < 0.05$; Table 8; Fig. 3B). The concentrations of glucose, triglycerides, total proteins, albumin, and globulins showed only a day effect ($P > 0.05$; Table 8).

The glucose concentration was reduced due to the age of the calves ($P < 0.05$; Fig. 4A). For triglycerides, a reduction was observed beginning at 210 days of calf age. The concentrations of total proteins, albumin, and globulins showed a decrease starting at 180 days of calf age ($P < 0.05$; Fig. 5A, 5B e 6).

4. DISCUSSION

Using forage as a basal component in the diet of beef cattle on pasture, Paulino et al. (2004) suggested that, to ensure satisfactory animal performance, 40 to 50 g/kg BW of pdDM should be provided; as the average of the present study was 197.3 g/kg BW, above the recommended level, thus demonstrating that the amount of forage did not compromise animal performance.

In the present study, there was an increasing response for DM and OM intake that occurred directly with the increasing supplementation level, resulting in an increase in animal performance. Nonetheless, the DMF (kg/day) did not decrease with the increase in supplementation, because animals in the 10 g/kg BW treatment group had a higher ADG, and consequently greater weight, which compensated for the forage intake. When the DMF (g/kg BW) was expressed, there was a reduction in pasture intake for the 10 g/kg BW treatment group. The increase in DM and OM intake can be explained by the fact that supplementation can prompt changes in ruminal metabolism, with the use of protein: energy supplements, and

thus, there is a greater intake of total DM due to the increase in the passage rate (Lazzarini et al., 2009; Sampaio et al., 2009).

Detmann et al. (2014a) suggested that the use of a protein:energy ratio (CP:DOM) would be more useful for understanding the metabolic effects of protein intake, as it more reliably indicates the animal's metabolic adequacy. Associations between protein:energy and forage intake under tropical conditions have been established by several authors (Costa et al., 2011; Detmann et al., 2014b; Egan, 1977; Panjaitan et al., 2010), with the response for maximum intake observed at 288 g CP/kg DOM (Detmann et al., 2014b).

Analysis of the intake of the treatments in the study showed that the 10 g/kg BW treatment allowed a ratio of 287.6 g CP/kg DOM, close to the ratio that Detmann et al. (2014b) suggested. According to Souza et al. (2010), this ratio reflects a higher intake, allows efficient use of protein, and enables greater availability of metabolizable energy and protein for animal metabolism for tissue synthesis. On the other hand, Lopes et al. (2017) observed a reduction in performance when supplementing the amount of 9.6 g/kg BW, for which the CP:MOD ratio of this level of supplementation was 326.9 g CP/kg DOM—that is, much higher than the 288 g PB/kg DOM suggested by Detmann et al. (2014b), thus showing a reduction of intake behavior and consequently reduced performance.

As the milk yield did not differ among treatments and decreased over time, it appears that milk intake would not interfere with the treatments. According to Carvalho et al. (2019), the type and amount of carbohydrates present in the diet can affect both the intake and digestibility of DM, NFC, and OM. Therefore, a higher intake of NFC, TDN, and DOM was observed with the increase in supplementation. That can be explained by the composition of the diets, in which corn and soybean meal showed high contents of OM and NFC, in addition to good palatability.

Microbial growth is affected by the availability of nutrients required by rumen microorganisms, such as carbohydrates, ammonia, peptides, amino acids, sulfur, and branched-chain fatty acids (Van Soest, 1994). As a result, the increase in DOM increased MPS. However, when evaluating the microbial efficiency (MPS/DOM) and the retained N/N intake (g/kg), these values maintained the same proportion, indicating that, for all treatments, there was a sufficient substrate for the development of the ruminal microbiota and good performance (Batista et al., 2016).

Serum concentrations of total protein and albumin are determined by animal protein metabolism and can be considered better indicators of animal status than BUN, since it has different concentrations during the day. Albumin is the main serum protein synthesized by the

liver, and its concentration can be influenced by the availability of amino acids and nutrients (da Silva et al., 2017). Contreras (2000) correlated this reduction in these metabolites to the growth period, when animals are kept on low protein pasture for a period of approximately four months. This reduction may be associated with the rainy-dry transition period and the beginning of the dry season, since during this time there is a drastic drop in the quality of grasses, due mainly to the reduction in CP contents (Detmann et al., 2014a). From 210 days of calf age, the pasture in this study began to show a drop in quality, reducing the CP and increasing the iNDF, and an accompanying reduction in milk production by the cows, thus reducing the concentrations of triglycerides and glucose from this period and leading to a drop in the concentrations of these protein metabolites.

Glucose concentration decreased with calf age, consistent with the results of Nemati et al. (2015) and de Khan et al. (2011). According to Hammon et al. (2002), this reduction in glucose concentrations may be attributed to physiological changes in the primary energy source from glucose to volatile fatty acids, as the calves' rumen increases its function with a higher solid diet intake. In the early stages of calf life, the cow has higher milk production and calves have higher blood glucose concentrations. After the third month of age, milk does not meet the calves' needs to support their potential growth (Costa e Silva et al., 2015; Valadares Filho et al., 2016), so there is a reduction in the intake of easily digested and absorbed nutrients, which leads to a decrease in circulating glucose as the calf's age increases until weaning (Reynolds et al., 2003).

The serum concentration of triglycerides is correlated to the absorption of lipids from the diet, mobilization from other tissues, and its use as an energy source (Kaneko et al., 2008); its reduction is related to lower milk intake. According to Jeshari et al. (2016), a reduction in triglyceride concentrations can be observed in calves that decrease milk intake over time. As a result, the animals show a higher intake of solid diet to sustain higher performance gains.

According to Valadares Filho et al. (2016), elements such as pasture quantity and quality of cows' milk production, calves' potential growth, breed, sex, calves' age at weaning, and even the type of supplement and the time of *creep-feeding* influence the animals' performance. Considering the limits imposed by genetics, the lower the ability of pasture and/or milk to supply the nutritional requirements of calves, the greater the relative response to *creep-feeding*, reflecting positively on the efficiency and profitability of this practice.

According to Paulino et al. (2014), to produce young animals on pasture, nutrients must be provided to guarantee an ADG of 0.9 kg/day for these animals to be slaughtered at 16 months of age. This performance was observed for all treatments evaluated. Because the

animals are genetically precocious and there is no lack of basal substrate in quantity and quality for both dams and calf, the relative response to the use of *creep-feeding* was reduced, being optimized only when higher levels of supplementation were used. For those animals that achieved higher performance, we also observed higher concentrations of IGF-1, because this hormone acts as a potent stimulator of cell proliferation and hypertrophy (Lawrence et al., 2012). In addition, an increase in IGF-1 concentrations was observed with increasing age of the calf, showing differences between treatments for the ADG and final BW of the animals: higher for animals in the 10 g/kg BW treatment compared to the two other treatments, which did not differ from one another.

However, animal growth is a quantitative measure related to the increase in body mass, which is related to tissue deposition of different structures and functions. According to Asher et al. (2018), this lower gain efficiency may be related to differences in gain composition, where in response to increased dietary energy content, fat deposition rates increase rather than muscle deposition rates. Gerrits et al. (1997) demonstrated that when the rate of fat deposition increases, the rate of protein deposition decreases, and thus the body weight gain decreases.

The muscle tissue of animals in the control treatment was not compromised by the lower nutrient intake, as observed by the rump depth. In contrast, according to Asher et al. (2018), it was observed that animals in the 5 and 10 g/kg BW treatment groups showed no differences in rib fat thickness and rump fat thickness, which may be related to the greater distribution in the deposition of body tissues of the supplemented animals. On the other hand, the animals in the control group showed less fat deposition in the rib and rump, especially after 210 days of age. With increasing age, the animals' requirements increase, the cows' milk production decreases, and the pasture begins to lose quality, with an increase in the iNDF of the forage. As a result, there is a lower intake of nutrients by these animals, limiting the tissue deposition, but not differing from the other treatments in terms of rump depth—that is, following the order of deposition: bone, muscle, and then adipose tissue.

Given the results obtained in this study, as it was a year with greater rainfall, forage presented good quality throughout the study, resulting in lower additional weight gain of supplemented animals in relation to non-supplemented animals, and greater performance, body composition and metabolic characteristics were higher for animals receiving 10 g/kg BW supplement. With this in mind, a supplementation cost worksheet was developed (worksheet attached) for decision making regarding the feasibility of supplementation, regardless of the region evaluated.

5. CONCLUSIONS

Under good quality pasture conditions, a supplement level of 10 g/kg BW containing 250 g BW/kg DM is recommended for suckling Nellore calves on tropical pastures. Supplementation increases dry matter intake, improves metabolic characteristics, body composition and performance of calves in the suckling phase.

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6. REFERENCES

- Adams, D.C., Nielsen, M.K., Schacht, W.H., Clark, R.T., 2000. Designing and conducting experiments for range beef cows. *Journal of Animal Science* 77, 1. <https://doi.org/10.2527/jas2000.77E-Suppl1u>
- Aguiar, A.D., Vendramini, J.M.B., Arthington, J.D., Sollenberger, L.E., Caputti, G., Sanchez, J.M.D., Cunha, O.F.R., da Silva, W.L., 2015. Limited creep-feeding supplementation effects on performance of beef cows and calves grazing limpograss pastures. *Livestock Science* 180, 129–133. <https://doi.org/10.1016/j.livsci.2015.08.008>
- Asher, A., Shabtay, A., Cohen-Zinder, M., Aharoni, Y., Miron, J., Agmon, R., Halachmi, I., Orlov, A., Haim, A., Tedeschi, L.O., Carstens, G.E., Johnson, K.A., Brosh, A., 2018. Consistency of feed efficiency ranking and mechanisms associated with inter-animal variation among growing calves. *Journal of Animal Science* 96, 990–1009. <https://doi.org/10.1093/jas/skx045>
- Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle. *Journal of Animal Science* 89, 510–519. <https://doi.org/10.2527/jas.2009-2366>
- Barros, L.V. de, Paulino, M.F., Marquez, D.S.C., Cabral, C.H.A., Silva, F.G. da, Caldeira, D.S.A., Lopes, S.A., Moura, F.H. de, 2015. Supplementation in a creep-feeding system for suckling beef calves and nutritional evaluation of beef sows. *Sem. Ci. Agr.* 36, 3431. <https://doi.org/10.5433/1679-0359.2015v36n5p3431>

- Batista, E.D., Detmann, E., Titgemeyer, E.C., Valadares Filho, S.C., Valadares, R.F.D., Prates, L.L., Rennó, L.N., Paulino, M.F., 2016. Effects of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nellore cattle fed low-quality tropical forage. *Journal of Animal Science* 94, 201–216. <https://doi.org/10.2527/jas.2015-9493>
- Carvalho, V.V., Paulino, M.F., Detmann, E., Filho, S.C.V., Lopes, S.A., Rennó, L.N., Sampaio, C.B., Silva, A.G., 2019. A meta-analysis of the effects of creep-feeding supplementation on performance and nutritional characteristics by beef calves grazing on tropical pastures. *Livestock Science* 227, 175–182. <https://doi.org/10.1016/j.livsci.2019.07.009>
- CEPEA/ USP - Center for Advanced Studies in Applied Economics, University of São Paulo. Agribusiness GDP - Agribusiness participation in Brazil's GDP. Available: <<https://www.cepea.esalq.usp.br/br/pib-do-agronegocio-brasileiro.aspx>>. Acesso em: 15 Jan. 2023 - CEPEA-USP/CNA, 2023.
- Contreras P. 2000. Indicators of protein metabolism used in metabolic profiles of herds. In: FHD G, Barcellos JO, Ospina H, LAO R, editors. *In Metabolic Profile in Ruminants: Its Use in Nutrition and Nutritional Diseases*, vol. 1. Porto Alegre: Printing at the Federal University of Rio Grande do Sul; p. 21–30.
- Costa e Silva, L.F., Engle, T.E., de Valadares Filho, S., Rotta, P.P., Villadiego, F.A.C., Silva, F.A.S., Martins, E.C., Silva, L.H.R., Paulino, M.F., 2015. Nellore cows and their calves during the lactation period: performance, intake, milk composition, and total apparent digestibility. *Trop Anim Health Prod* 47, 735–741. <https://doi.org/10.1007/s11250-015-0787-6>
- Costa, V.A.C., Detmann, E., Paulino, M.F., Valadares Filho, S. de C., Carvalho, I.P.C. de, Monteiro, L.P., 2011. Intake and digestibility in grazing cattle during the rainy season supplemented with sources of nitrogen compounds and carbohydrates. *R. Bras. Zootec.* 40, 1788–1798. <https://doi.org/10.1590/S1516-35982011000800024>
- Cremin, J.D., Jr., Faulkner, D.B., Merchen, N.R., Fahey, G.C., Jr., Fernando, R.L., Willms, C.L., 1991. Digestion criteria in nursing beef calves supplemented with limited levels of protein and energy. *Journal of Animal Science* 69, 1322–1331. <https://doi.org/10.2527/1991.6931322x>
- 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., 2017. Performance, endocrine, metabolic, and reproductive responses of Nellore heifers

- submitted to different supplementation levels pre- and post-weaning. *Trop Anim Health Prod* 49, 707–715. <https://doi.org/10.1007/s11250-017-1248-1>
- Detmann, E., Paulino, M.F., Zervoudakis, J.T., Valadares Filho, S. de C., Euclides, R.F., Lana, R. de P., Queiroz, D.S. de, 2001. Chromium and internal indicators in determining the intake of supplemented crossbred steers on pasture. *R. Bras. Zootec.* 30, 1600–1609. <https://doi.org/10.1590/S1516-35982001000600030>
- Detmann, E., Paulino, M.F., Valadares filho, S.C. 2010. Optimization of the use of basal forage resources. In: *Proceedings of the 7th Beef Cattle Production Symposium*, Viçosa. p.191-240.
- Detmann E, Paulino MF, Valadares Filho SDC, Huhtanen P, 2014a. Nutritional aspects applied to grazing cattle in the tropics: a review based on Brazilian results. *Sem. Ci. Agr.* 35, 2829. <https://doi.org/10.5433/1679-0359.2014v35n4Suplp2829>
- Detmann, E., Valente, É.E.L., Batista, E.D., Huhtanen, P., 2014b. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. *Livestock Science* 162, 141–153. <https://doi.org/10.1016/j.livsci.2014.01.029>
- Detmann, E; Costa e Silva, L.F; Palma, M.N.N; Rocha, C.R. 2021. *Methods for food analysis – INCT – Animal Science*. 2nd edition. Viscount of Rio Branco. Supreme: Brazil. P. 350.
- Egan, A.R., 1977. Nutritional status and intake regulation in sheep. VIII.* Relationships between voluntary intake of herbage by sheep and the protein/energy ratio in the digestion products. *Aust. J. Agric. Res.* 28, 907–915. <https://doi.org/10.1071/ar9770907>
- Faulkner, D.B., Hummel, D.F., Buskirk, D.D., Berger, L.L., Parrett, D.F., Cmarik, G.F., 1994. Performance and nutrient metabolism by nursing calves supplemented with limited or unlimited corn or soyhulls. *Journal of Animal Science* 72, 470–477. <https://doi.org/10.2527/1994.722470x>
- Gerrits, W.J.J., France, J., Dijkstra, J., Bosch, M.W., Tolman, G.H., Tamminga, S., 1997. Evaluation of a Model Integrating Protein and Energy Metabolism in Preruminant Calves. *The Journal of Nutrition* 127, 1243–1252. <https://doi.org/10.1093/jn/127.6.1243>
- Hammon, H.M., Schiessler, G., Nussbaum, A., Blum, J.W., 2002. Feed Intake Patterns, Growth Performance, and Metabolic and Endocrine Traits in Calves Fed Unlimited Amounts of Colostrum and Milk by Automate, Starting in the Neonatal Period.

- Journal of Dairy Science 85, 3352–3362. [https://doi.org/10.3168/jds.S0022-0302\(02\)74423-8](https://doi.org/10.3168/jds.S0022-0302(02)74423-8)
- Jeshari, M., Riasi, A., Mahdavi, A.H., Khorvash, M., Ahmadi, F., 2016. Effect of essential oils and distillation residues blends on growth performance and blood metabolites of Holstein calves weaned gradually or abruptly. *Livestock Science* 185, 117–122. <https://doi.org/10.1016/j.livsci.2015.12.011>
- Khan, M.A., Weary, D.M., von Keyserlingk, M.A.G., 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. *Journal of Dairy Science* 94, 3547–3553. <https://doi.org/10.3168/jds.2010-3871>
- Lawrence, T. J. J.; Fowler, V. R.; Novakofski, J. E. 2012. *Growth of farm animals*. 3 ed. CABI Publishing: London, UK, 368p.
- Lazzarini, I.; Detmann, E.; Sampaio, C. B., Paulino, M.F. 2009. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Rev. Brasileira de Zootecnia*, v. 38, p. 2021-2030. <https://doi.org/10.1590/S1516-35982009001000024>
- 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., 2017. Evaluation of supplementation plans for suckling beef calves managed on tropical pasture. *SCA* 38, 1027. <https://doi.org/10.5433/1679-0359.2017v38n2p1027>
- Nemati, M., Amanlou, H., Khorvash, M., Moshiri, B., Mirzaei, M., Khan, M.A., Ghaffari, M.H., 2015. Rumen fermentation, blood metabolites, and growth performance of calves during transition from liquid to solid feed: Effects of dietary level and particle size of alfalfa hay. *Journal of Dairy Science* 98, 7131–7141. <https://doi.org/10.3168/jds.2014-9144>
- NRC, 2016. *National Research Council, Nutrient Requirement of Beef cattle*, 8th edition. National Academy Press, Washington, DC. Owens, F.N., Dubeski, P., Hanson, C.F., 1993. Factors that alter the growth and development of ruminants. *J. Anim. Sci.* 71 (11), 3138–3150.
- Panjaitan, T., Quigley, S.P., McLennan, S.R., Swain, T., Poppi, D.P., 2010. Intake, retention time in the rumen and microbial protein production of *Bos indicus* steers consuming grasses varying in crude protein content. *Anim. Prod. Sci.* 50, 444. <https://doi.org/10.1071/AN09197>

- Paulino, M. F.; Figueiredo, D. M.; Moraes, E. H. B. K. 2004. Supplementation of cattle on pasture: a systemic view. In: Symposium on beef cattle production, 4., Viçosa, MG. Anais. Viçosa, MG: Simcorte. pp. 93-129.
- Paulino, M. F, Detmann, E, Valente E. E. L. 2008. Nutrition of grazing cattle. In: Symposium on Strategic Pasture Management. Viçosa, MG. Annals. Viçosa, MG: Simcorte. pp. 131–169.
- Paulino, M. F.; Detmann, E.; Silva, A. G. et al. 2014. Optimized cattle breeding In: Symposium on beef cattle production, 9, Viçosa, MG. Annals. Viçosa, MG: Simcorte. pp. 139-164.
- Reynolds, C.K., Aikman, P.C., Lupoli, B., Humphries, D.J., Beever, D.E., 2003. Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. *Journal of Dairy Science* 86, 1201–1217. [https://doi.org/10.3168/jds.S0022-0302\(03\)73704-7](https://doi.org/10.3168/jds.S0022-0302(03)73704-7)
- Sampaio, C.B., Detmann, E., Lazzarini, I., Souza, M.A. de, Paulino, M.F., Valadares Filho, S. de C., 2009. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *R. Bras. Zootec.* 38, 560–569. <https://doi.org/10.1590/S1516-35982009000300023>
- Silva Júnior, J.M., Rodrigues, J.P.P., Valadares Filho, S. de C., Detmann, E., Paulino, M.F., Rennó, L.N., 2021. Estimating purine derivatives and nitrogen compound excretion using total urine collection or spot urine samples in grazing heifers. *J Anim Physiol Anim Nutr* 105, 861–873. <https://doi.org/10.1111/jpn.13525>
- Souza, M.A., Detmann, E., Paulino, M.F., Sampaio, C.B., Lazzarini, Í., Valadares Filho, S.C., 2010. Intake, digestibility and rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogen and/or starch. *Trop Anim Health Prod* 42, 1299–1310. <https://doi.org/10.1007/s11250-010-9566-6>
- Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H., Löest, C.A., 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. *Journal of Animal Science* 79, 1059–1063. <https://doi.org/10.2527/2001.7941059x>
- 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., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: *Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE*. Editora Federal de Viçosa. <https://doi.org/10.5935/978-85-8179-111-1.2016B002>

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2th ed. Ithaca: Cornell University Press. 476p.

7. TABLES

Table 1
Supplement ingredients (g/kg)

Ingredients	g/kg
Corn meal	560
Soybean meal	438.5
Cremaron (Phytobiotics) ¹	1.5

¹ Palatabilizer

Table 2
Chemical composition of the supplement and forage

Items ¹	Supplement ⁴	<i>U. decumbens</i>					
		February	March	Digestibility	June	Mean	
DM ²	893.4	253.5 ± 0.53	265.5 ± 0.33	261.2 ± 0.64	270.1 ± 1.44	262.575	±0.87
OM ³	963.0	899.3 ± 0.15	903.5 ± 0.21	918.6 ± 0.25	904.8 ± 0.36	906.55	±0.25
CP ³	257.3	112.3 ± 0.05	109.3 ± 0.02	111.1 ± 0.03	93.90 ± 0.37	106.65	±0.15
EE ³	34.9	10.5 ± 0.04	11.2 ± 0.02	10.5 ± 0.03	10.5 ± 0.02	10.675	±0.02
NFC ³	558.5	168.8 ± 0.61	157.9 ± 0.31	221.3 ± 0.58	182.3 ± 0.50	182.575	±0.56
apNDF ³	112.4	617.9 ± 0.72	622.4 ± 0.12	588.1 ± 0.64	618.1 ± 0.43	611.625	±0.59
iNDF ³	20.0	158.9 ± 0.34	159.7 ± 0.25	162.0 ± 0.28	217.1 ± 0.58	174.425	±0.40

¹ DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NFC = non-fiber carbohydrates; apNDF = neutral detergent fiber corrected for ash and protein; iNDF = indigestible NDF.

² In g/kg of natural matter.

³ In g/kg of dry matter.

⁴ Samples obtained from the supplement during the experimental period, respectively.

Table 3
Intake of lactating beef calves according to different levels of supplementation

Items ¹	Treatments (g/kg BW)			SEM	P-Value ²
	0	5	10		
	kg/day				
DM	3.07c	3.96b	4.68a	0.131	0.001
DMF	2.35	2.25	1.90	0.06	0.113
DMS	0c	0.95b	1.97a	0.038	< 0.001
DMM	0.72	0.76	0.81	0.078	0.712
OM	2.81c	3.67b	4.39a	0.126	< 0.001
CP	0.45c	0.69b	0.93a	0.035	< 0.001
EE	0.27	0.32	0.37	0.04	0.155
TDN	2.07c	2.81b	3.6a	0.123	< 0.001
apNDF	1.39	1.44	1.31	0.058	0.302
NFC	0.70c	1.22b	1.78a	1.069	< 0.001
DOM	1.79c	2.5b	3.23a	0.111	< 0.001
iNDF	0.39	0.38	0.34	0.025	0.131
dNDF	0.82	0.89	0.77	0.022	0.22
CP:DOM(g/Kg)	253.2c	278.3b	287.6a	4.551	0.005
	g/kg BW				
DM	15.2c	18.8b	19.9a	0.422	0.002
DMF	11.6a	10.65a	8.07b	0.319	0.001
OM	13.94c	17.57b	18.71a	0.411	0.001
apNDF	6.87a	6.89a	5.6b	0.231	0.009
iNDF	1.97a	1.82a	1.44b	0.128	0.002

¹ DM = total dry matter intake; DMF = dry matter of forage intake; DMS = dry matter intake of supplement DMM = milk dry matter intake; OM = organic matter; CP = crude protein; EE = ether extract; TDN = total digestible nutrients; NFC = non-fiber carbohydrates; apNDF = neutral detergent fiber corrected for ash and protein; iNDF = indigestible NDF; dOM = digested organic matter; dNDF = digested NDF.

² Means with different lowercase letters in the row differ at 5% probability level by t test.

Table 4
Average milk production and composition of cows

Items	Treatments (g/kg BW)			SEM	<i>P</i> -Value ¹
	0	5	10		
Production, kg/dia	5.9	6.3	6.6	0.623	0.645
Protein, %	4.3	4.2	4.1	0.163	0.854
Fat, %	5.1	5.2	5.3	0.256	0.772
Lactose, %	4.6	4.6	4.6	0.084	0.838
Total solids, %	14.9	15.0	15.0	0.376	0.954

¹ Means with different lowercase letters in the row differ at 5% probability level by t test.

Table 5
Digestibility coefficient (kg/kg) of lactating beef calves according to different levels of supplementation

Item ¹	Treatments (g/kg BW)			SEM	<i>P</i> -Value ³
	0	5	10		
DM	0.60c	0.63b	0.69a	0.012	0.006
OM	0.64c	0.68b	0.73a	0.008	0.002
CP	0.86b	0.87ab	0.89a	0.004	0.027
EE	0.79	0.78	0.80	0.025	0.829
apNDF	0.59	0.61	0.59	0.016	0.359
NFC	0.51c	0.61b	0.74a	0.028	0.004
DOM (g/Kg DM)	581c	632b	689a	9.06	0.029

¹ DM = total dry matter intake; OM = organic matter; CP = crude protein; EE = ether extract; NFC = non-fiber carbohydrate; apNDF = neutral detergent fiber corrected for ash and protein; DOM = digested organic matter.

² Means with different lowercase letters in the row differ at 5% probability level by t test.

Table 6

Synthesis of nitrogenous compounds of lactating beef calves according to different levels of supplementation

Items ¹	Treatments (g/kg BW)			SEM	P-Value ²
	0	5	10		
N intake (g/d)	72.5c	111.1b	149.8a	4.191	<0.001
Fecal N (g/d)	10.1b	14.0a	16.0a	0.916	0.018
Urine N (g/d)	34.5c	63.8b	72.8a	4.454	0.003
Urea N (g/d)	23.4b	42.8a	49.6a	2.375	0.001
Retained N (g/d)	27.9b	36.6b	61.1a	4.896	0.008
MPS (g/d)	198.5b	225.7b	395.9a	35.56	0.014
RUP (g/d)	254.5b	468.8a	541.1a	39.38	0.007
Retained N/N intake (g/kg)	383.1	317.0	407.8	38.62	0.244
MP (g/d)	330.9c	519.2b	686.0a	20.77	<0.001
MNS (g/g N intake)	0.44	0.33	0.42	0.047	0.254
RUP/CP intake (g/kg)	555.6	666.0	580.0	47.87	0.259
MPS/DOM (g/kg)	112.9	92.6	120.9	14.32	0.329

¹ MNS = microbial nitrogen synthesis: nitrogen intake; MPS = microbial protein synthesis; RUP = rumen undegradable protein; MP = metabolizable protein; CP = crude protein; DOM = digestible organic matter.

² Means with different lowercase letters in the row differ at 5% probability level by t test.

Table 7

Performance and carcass characteristics of beef cows and calves according to different levels of supplementation

Items ¹	Treatments (g/kg BW)			SEM	P-Value ²		
	0	5	10		Trat	Day ³	Trat x Day
Cows							
Initial BW, kg	523.6	485.2	512.2	20.29	0.573	-	-
Final BW, kg	568.3	538.1	561.2	25.79	0.642	-	-
ADG, kg/d	0.29	0.29	0.33	0.055	0.819	-	-
Initial BCS	5.3	5.4	5.6	0.162	0.516	-	-
Final BCS	6.4	6.3	6.7	0.277	0.407	-	-
Calves							
Initial BW, kg	114.0	115.1	117.5	6.017	0.394	-	-
Final BW, kg	239.8b	252.9b	283.9a	5.21	0.041	-	-
ADG, kg/d	0.90b	0.98b	1.19a	0.038	0.001	-	-
Rib-eye area, cm ²	31.7	36.2	36.6	1.538	0.093	<0.001	0.017
Rump depth ,cm	57.2b	60.7ab	62.4a	1.164	0.031	<0.001	0.785
Rib fat thickness ,mm	0.99b	1.24a	1.31a	0.074	0.018	<0.001	0.007
Rump fat thickness ,mm	1.31b	1.57ab	1.72a	0.085	0.02	<0.001	0.321

¹ BW = body weight; ADG = average daily gain; BCS = body condition score;

² Means with different lowercase letters in the row differ at 5% probability level by t test.

³ Day of birth.

Table 8
Metabolic profile of lactating beef calves according to different levels of supplementation

Items ¹	Treatments (g/kg BW)			SEM	P-Value ²		
	0	5	10		Trat	Day ³	Trat x Day
Glucose, mg/dL	83.4	84.7	89.9	2.693	0.209	<0.001	0.675
IGF-1, ng/mL	267.4b	293.7b	333.2a	15.740	0.049	<0.001	0.882
BUN, mg/dL	11.8b	17.1a	19.3a	1.312	0.018	<0.001	0.662
Triglycerides, mg/dL	36.9	37.7	35.9	4.822	0.949	<0.001	0.788
Total proteins, g/dL	5.1	5.3	5.1	0.149	0.542	<0.001	0.784
Albumin, g/dL	2.95	3.19	3.04	0.076	0.131	<0.001	0.757
Globulins, g/dL	2.1	2.1	2.0	0.093	0.797	<0.001	0.878

¹ IGF-1 = insulin like growth factor; BUN = blood urea nitrogen.

² Means with different lowercase letters in the row differ at 5% probability level by t test.

³ Day of birth.

8. FIGURES

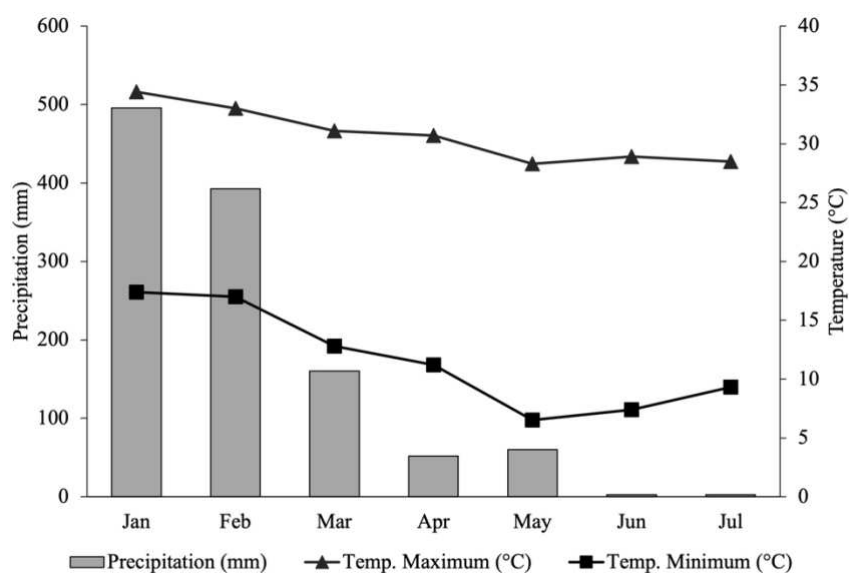
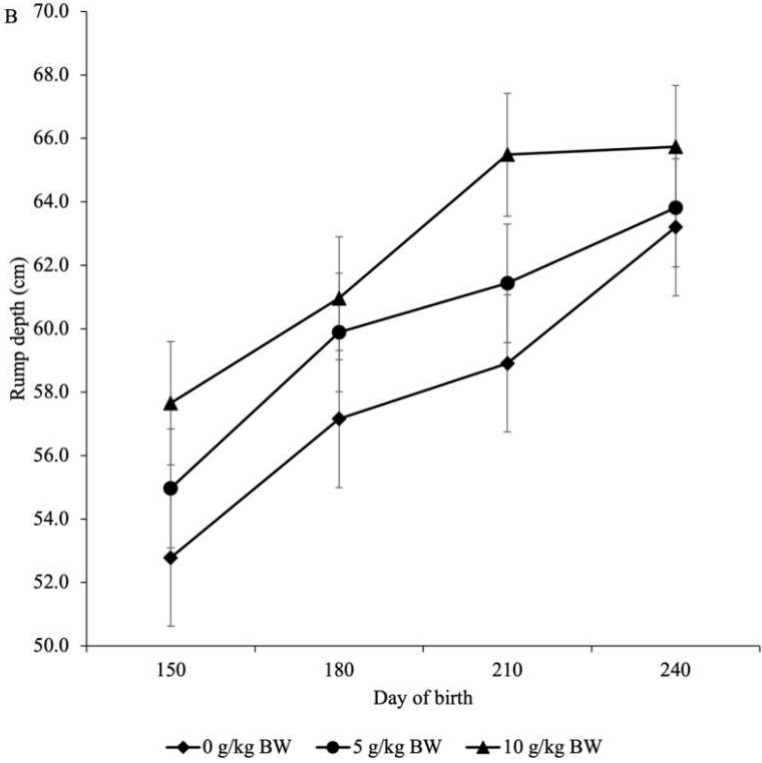
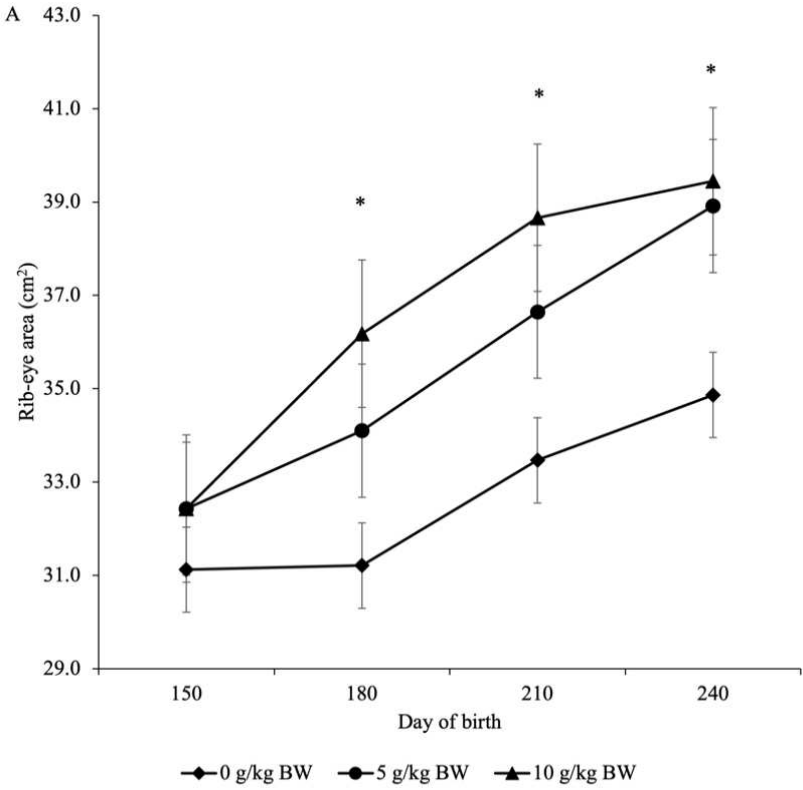


Fig 1. Precipitation and average temperature during the experimental period. Viçosa - MG.
Source: INMET



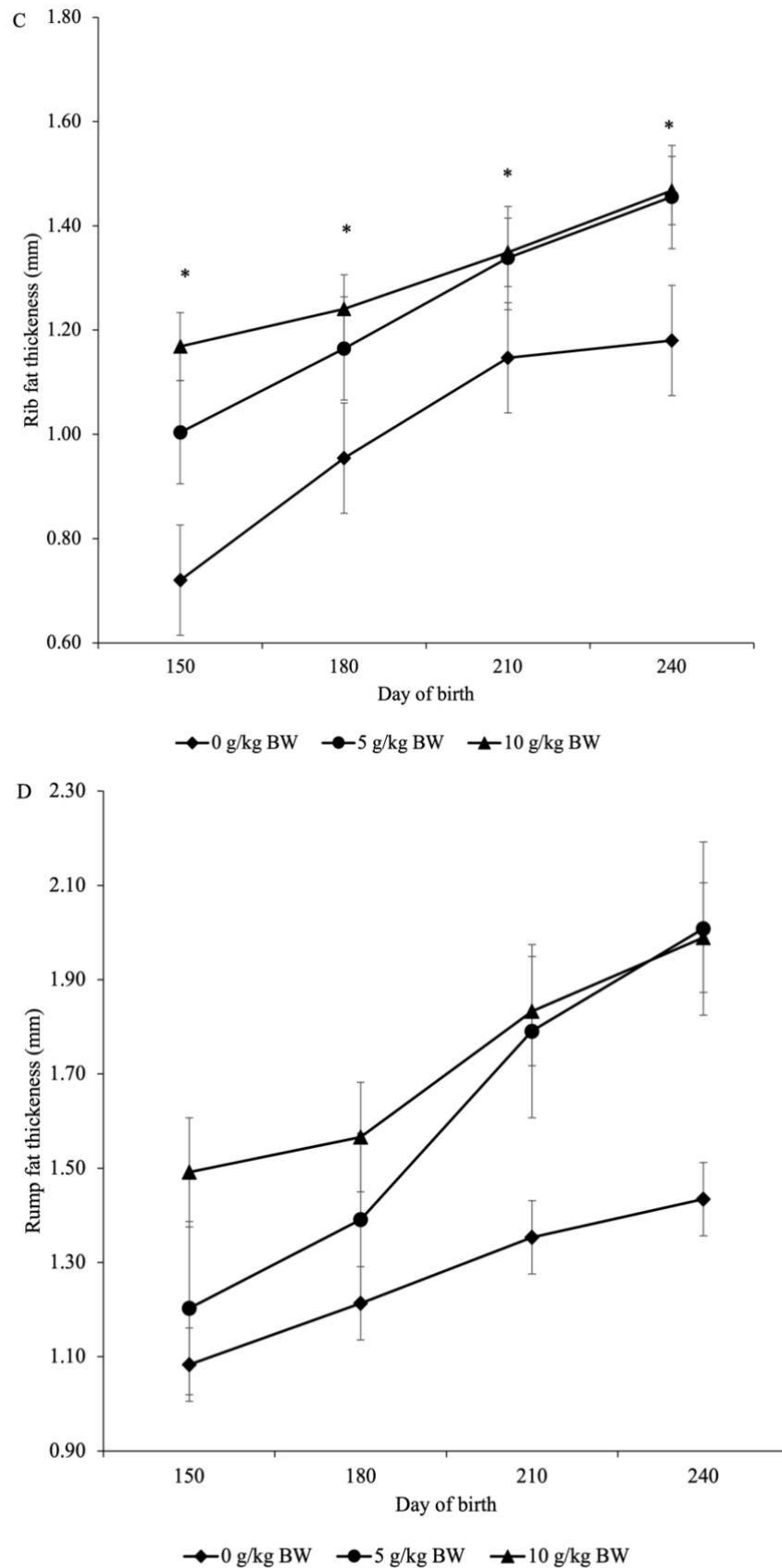


Fig 2. Carcass characteristics of beef calves supplemented on tropical pastures. 2A: Rib-eye area; 2B: Rump depth; 2C: 12th rib fat thickness; 2D: Rump fat thickness. Days with an asterisk (*) are significantly different between treatments (P < 0.05).

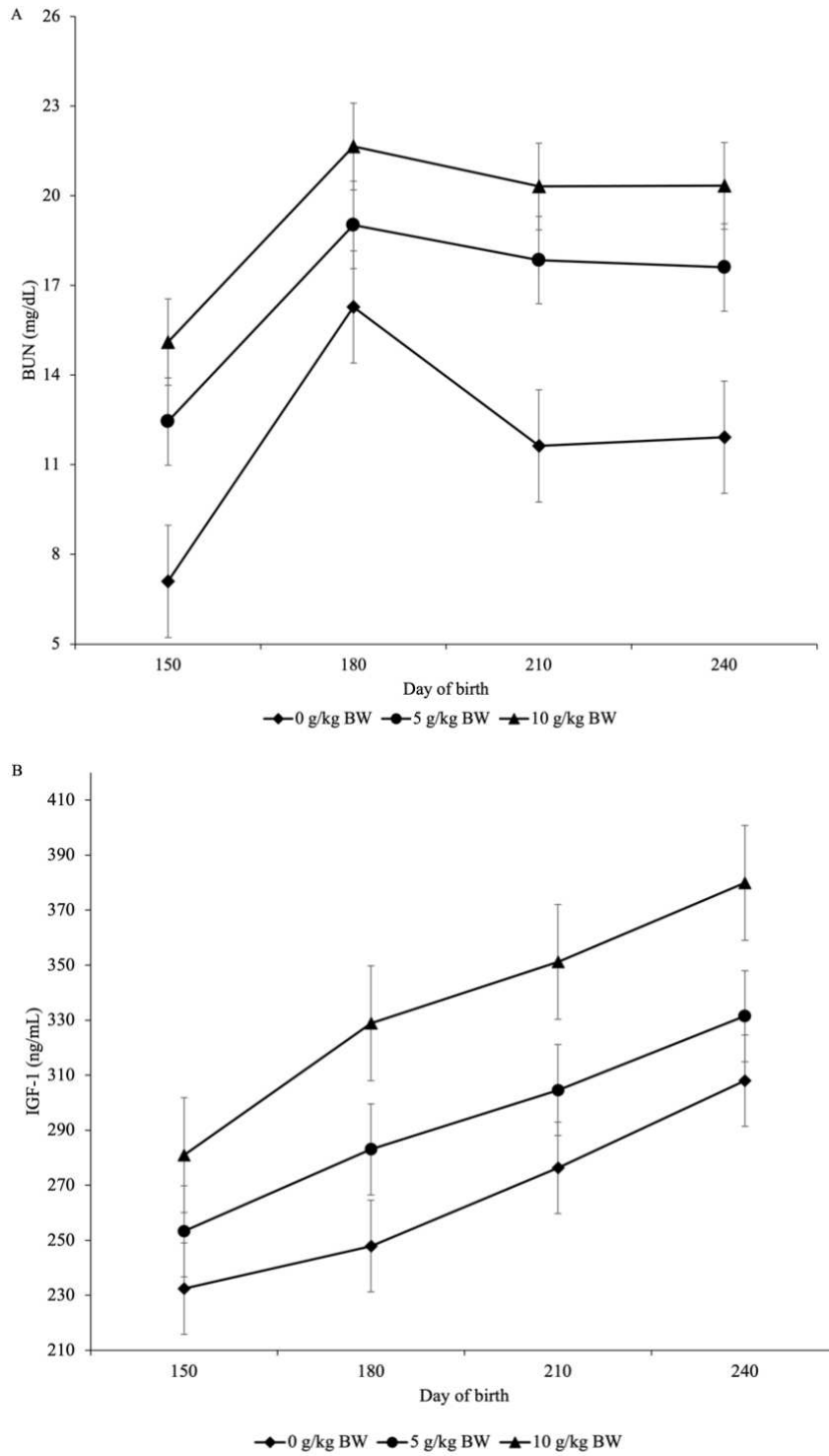


Fig 3. Concentrations of blood metabolites. 3A: Blood urea nitrogen (BUN); 3B: Insulin-like growth factor type 1 (IGF-1).

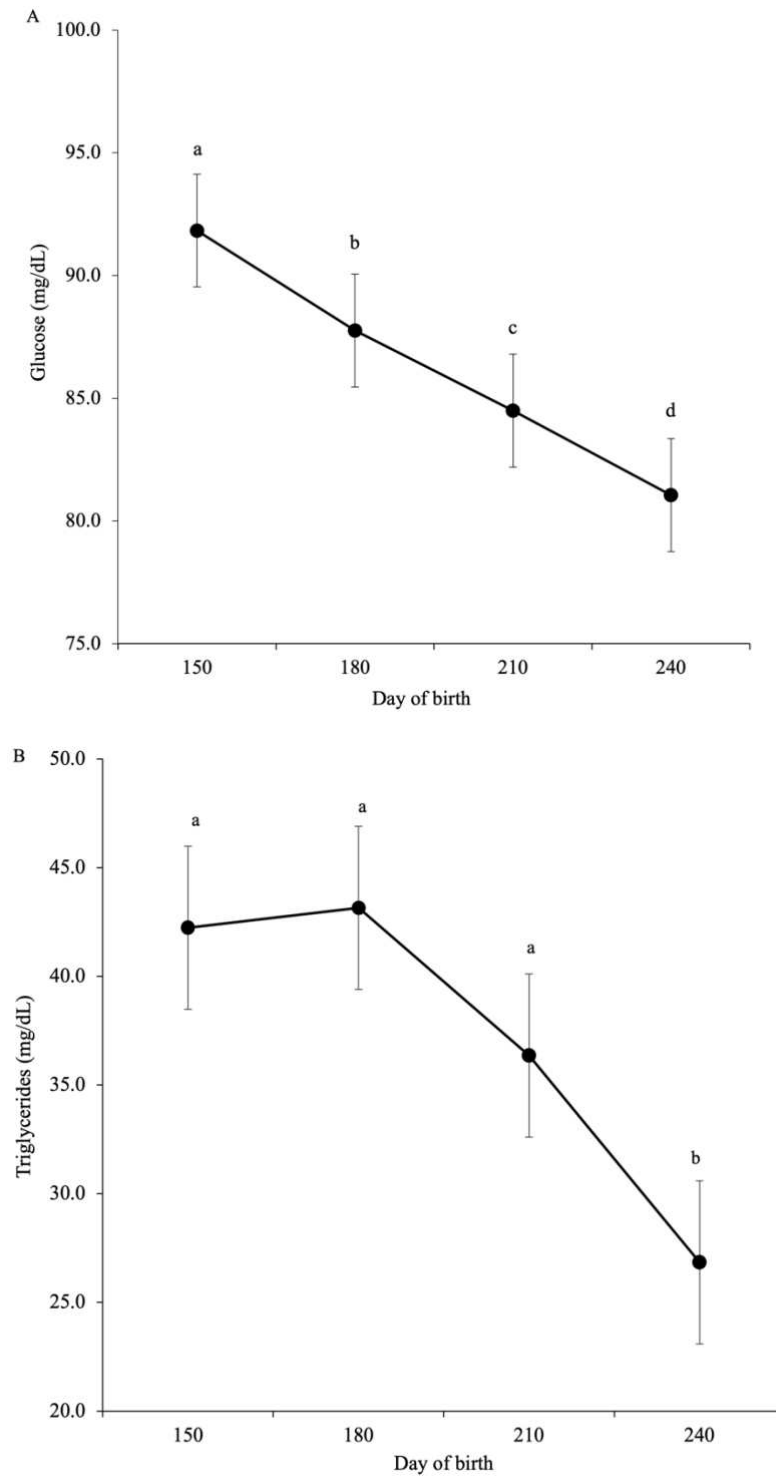


Fig 4. Concentrations of blood metabolites. 4A: Glucose; 4B: Triglycerides. Means with different lowercase letters differ at 5% probability level by t-test.

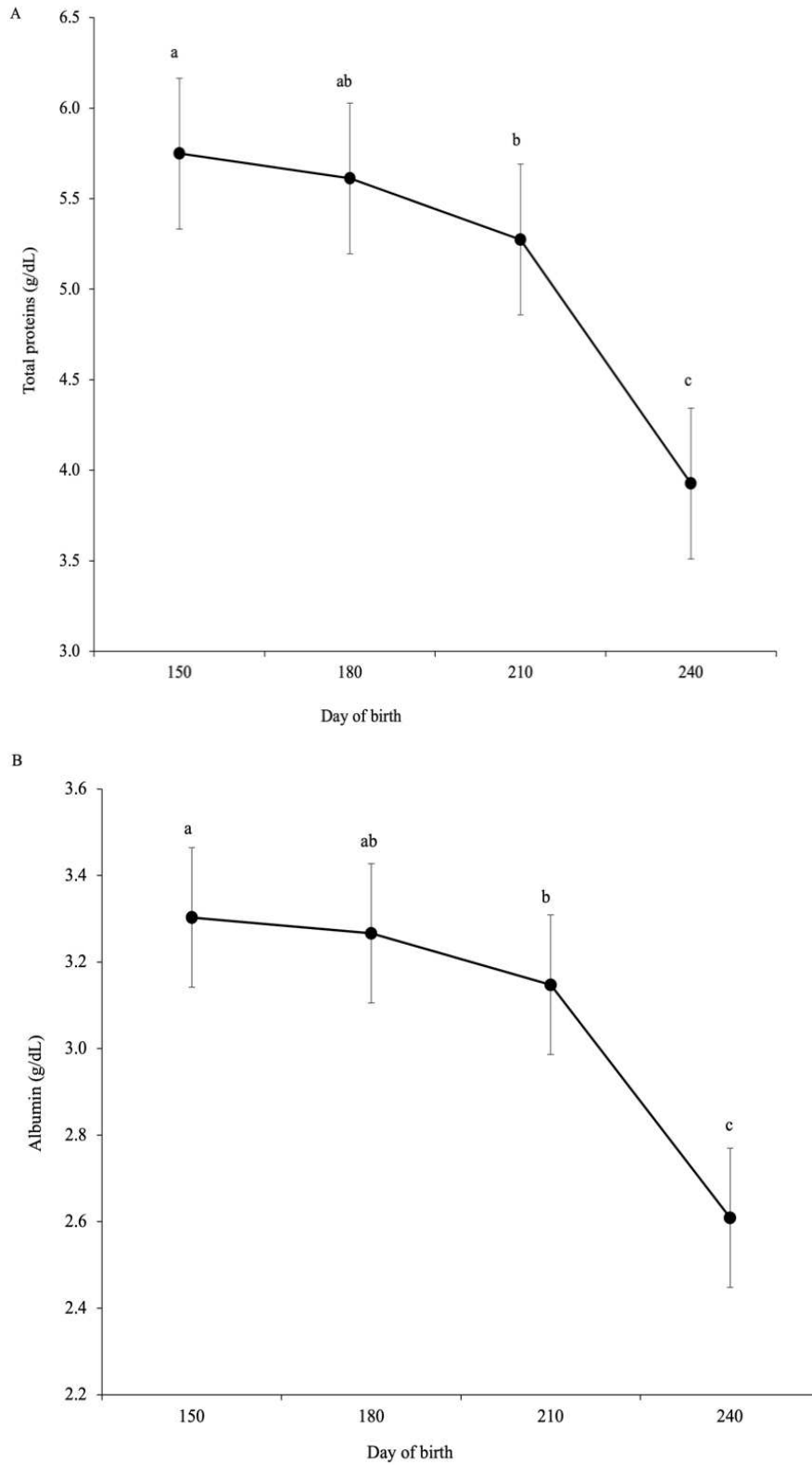


Fig 5. Concentrations of blood metabolites. 5A: Total proteins; 5B: Albumin. Means with different lowercase letters differ at 5% probability level by t-test.

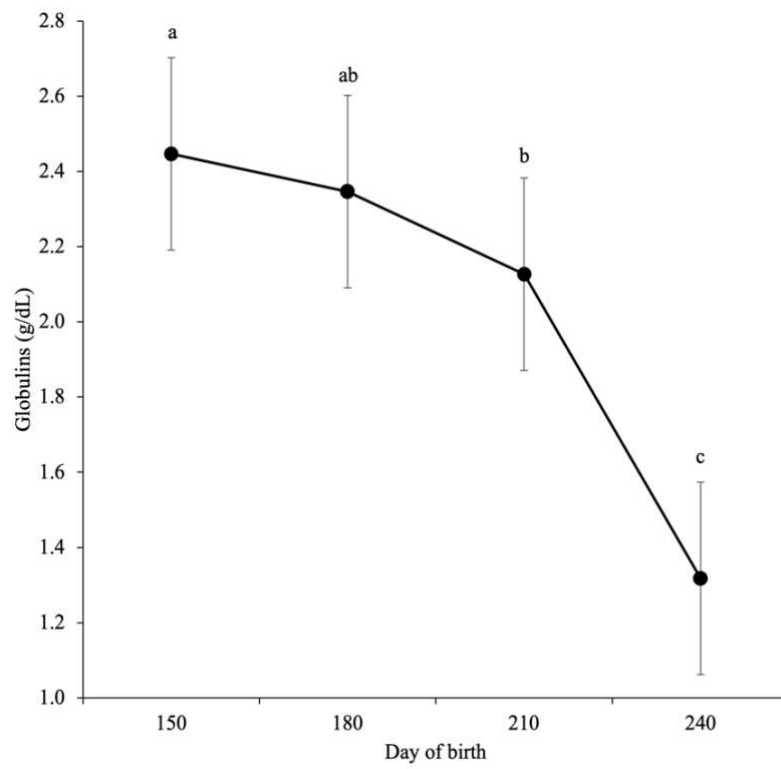


Fig 6. Globulin concentrations in the blood. Means with different lowercase letters differ at 5% probability level by t-test.

MANUSCRIPT 2: PERFORMANCE AND METABOLIC RESPONSES OF NELLORE COWS SUBJECTED TO DIFFERENT SUPPLEMENTATION PLANS DURING PREPARTUM

ABSTRACT

The objective of this study was to evaluate the effects of different supplementation plans for Nellore cows 60 days prepartum on performance, metabolic responses and influence on the initial body development of the offspring. We used 39 pluriparous, pregnant male Nellore cows with an initial mean of 224 ± 2.6 days of pregnancy, 520 ± 15.2 kg BW and 6.0 ± 0.07 (scale of 1 - 9) body condition score (BCS), according to completely randomized design, with four treatments and two repetitions. The treatments evaluated were: control receiving only mineral mixture *ad libitum*, 2 g/kg BW, 4 g/kg BW and 6 g/kg BW of protein-energy supplement per cow per day for 60 days before calving. The supplement was formulated to contain similar amounts of crude protein, 300 g CP/kg in DM. Statistical evaluations were conducted using the PROC MIXED procedure of SAS (version 9.4), adopting $\alpha = 0.05$. Cows supplemented with 4 and 6 g/kg BW presented greater BW variation in the prepartum and less variation in the postpartum ($P < 0.05$). There was a higher BCS for supplemented animals ($P < 0.05$) at calving and at 90 days postpartum, with animals in the 4 and 6 g/kg BW treatment being higher than the control treatment ($P < 0.05$). There were no differences in calf birth weight and performance up to 90 days postpartum ($P > 0.05$). Animals receiving 4 and 6 g/kg BW had a shorter service period compared to the control treatment ($P < 0.05$), but there were no differences for overall pregnancy outcome ($P > 0.05$). Treatment x day interaction was observed for BUN which was higher in the prepartum for animals that received higher supplementation levels ($P < 0.05$), β HB, was higher in the prepartum for animals in the control treatment ($P < 0.05$), and for NEFA, concentrations were lower 21 days before calving and at 21 and 42 days after calving, for the treatments that received 4 and 6 g/kg BW ($P < 0.05$). Progesterone concentrations presented a positive linear effect with the increase of supplement supply ($P < 0.05$), being higher for animals that received 4 and 6 g/kg BW compared to animals in the control treatment ($P < 0.05$). In view of these results, Providing 4g/kg BW of protein-energy supplement to grazing Nellore cows 60 days prior to calving is recommended, which improves metabolic characteristics and performance in the prepartum and postpartum period and a lower negative energy balance in the postpartum period, resulting in a shorter service period.

Keywords: Nutrition, Metabolism, Reproduction, NEFA, Progesterone.

1. INTRODUCTION

The nutritional status of beef cows at calving is the main factor influencing the service period (Baruselli et al., 2004; Mulliniks et al., 2013). Inadequate nutrient supply in late pregnancy impairs reproduction, even when this is sufficiently offered during lactation. There are studies showing that supplementation of cows in the prepartum period is more important than postpartum (Hess et al., 2005; Diskin et al., 2016; da Silva et al., 2017).

In beef cattle production systems, the breeding season occurs when production and nutritional level are optimal, however, beef cows spend most of their pregnancy period during the dry season, which is characterized by low availability and quality of forage. Thus, supplementation during this period can correct nutritional deficiencies and improve the body condition of cows (Paulino et al., 2010; Detmann et al., 2014), as this is one of the most important factors in determining the extent of postpartum anestrus (Ayres et al., 2014). On the other hand, excess nutrients in the prepartum period has also been associated with adverse effects on reproductive efficiency (Santos et al., 2008; Sartori et al., 2010; Wiltbank et al., 2006) and increased incidence of dystocia calving.

Changes in the nutritional status of beef cattle can be assessed through metabolites that link nutrition and physiology (Payne, 1987) and help to accurately indicate the effects of supplementation on animal metabolism. However, studies with *Bos indicus* under pasture condition are scarce and the results have been inconsistent (Lana Ferreira et al., 2020; De Almeida, et al., 2020). The hypothesis is that higher levels of supplementation in the prepartum period reduce the negative energetic balance and improve reproduction in Nellore cows. Thus, this study aimed to evaluate different nutritional plans for beef cows in prepartum and its effects on performance, metabolism and influence on the early development of the calves.

2. MATERIAL AND METHODS

2.1. Animals, experimental design and treatments

All practices involving the use of animals were approved by the Ethics Committee on Animal Use of the Federal University of Viçosa (Protocol CEUAP-UFV 142/19).

The experiment was conducted at the Teaching, Research and Extension Unit in Cattle (UEPE-GC), at the Federal University of Viçosa, Minas Gerais, Brazil (20 ° 45 ' S 42 ° 52 '

W). The research was conducted from July 2019 to January 2020. The mean values of temperature and precipitation were 22.5°C and 1295 mm (Figure 1).

We used 39 pluriparous, pregnant male Nelore cows with 224 ± 2.67 days pregnancy at the start of supplementation, 5.3 ± 0.29 years of age, body weight (BW) 520 ± 15.2 kg and mean initial body condition score (BCS) 6.0 ± 0.07 (scale of 1 - 9).

Prior to the experimental period, all animals composed a single lot, managed in a 30 ha area covered by *Urochloa decumbens*, with water troughs, shaded areas and troughs for the *ad libitum* supply of mineral mixture. To do so, the animals underwent 14 days of adaptation to the experimental area, to divisions into lots with 4 or 5 animals each, and also to the diet, receiving 2 g/kg BW of supplement. During the adaptation period, endo- and ectoparasites were also controlled with doramectin 3.5% (Treo ACE®, Zoetis, SP, Brazil).

The cows were randomly distributed in an experimental area consisting of eight paddocks in an entirely randomized design, where a group of four or five animals was considered the experimental unit. The paddocks were approximately 7.5 hectares each, covered with *Urochloa decumbens* grass, with free access to water and feeders.

The nutritional plans evaluated were: control, cows receiving only mineral mix *ad libitum*; 2, 4, or 6 = cows receiving 2, 4, or 6 g/kg BW, of protein-energy supplement for 60 days prepartum, plus mineral mix *ad libitum*. After calving, all groups received only mineral mixture *ad libitum* until 90 days postpartum. The supplement was formulated to contain 300 g CP/kg DM; (Tables 1 and 2).

Supplements were provided daily at 11:00 am. To prevent paddock effects in the treatments the animals were alternated between paddocks every seven days, so that each group remained for the same amount of time in each paddock.

2.2. Performance

To evaluate the performance of the cows, weights and BCS evaluation were performed at the beginning of the experiment (60 days prepartum) and 7 days before the expected calving date, and at 45 and 90 days after calving, the BCS were evaluated by 3 experienced evaluators, using the scale from 1 to 9 according to NRC (2016). Body weight variation at prepartum was performed being the difference in BW 7 days before calving compared to the initial weight and weight variation at postpartum being the difference in BW at 45 and 90 days compared to calving. The calves, soon after birth, received first treatments of navel healing, identification, and weighing. Weighing also occurred at 90 days after birth to evaluate the average daily gain (ADG).

The cows, 45 days after calving, underwent the mating season, during which they were synchronized and then performed fixed-time artificial insemination (FTAI). The same protocol was used for all animals, which consisted of the insertion of an intravaginal device containing 1g of progesterone (P4) (PRIMER®, Agener Union animal health, SP, Brazil) on the first day of the protocol (D0), together with the administration of 2 mg of estradiol benzoate (RIC BE®, Agener União animal health, SP, Brazil), via the intramuscular administration. After 8 days (D8), the intravaginally P4 device was removed and 0.524 mg of Cloprostenol (ESTRON®, Agener Union Animal health, SP, Brazil) and 300UI of equine chorionic gonadotropin (ECEGON®, Biogénesis Bagó animal health, Curitiba, Brazil), intramuscular. On day 9 (D9) we applied 1mg of estradiol benzoate (RIC BE®, Agener Union animal health, SP, Brazil), also via intramuscular.

Artificial insemination (AI) was scheduled according to preovulatory follicle diameter (PFOD) for 48 or 56 hours after P4 device removal. Animals with PFOD ≥ 14 mm at D10 were inseminated at the same time (48h), while those with PFOD < 14 mm were evaluated again after 8 hours, when they were then inseminated (56h), provided that follicular growth or ovulation was observed. Animals that presented PFOD less than 11.5 mm or more than 20 mm in any of the evaluations received 25 μ g of Lecirelina, a GnRH analog (TEC-Relin®, Agener Union animal health, SP, Brazil). 30 days after the FTAI, pregnancy was diagnosed and those that were not pregnant underwent a second FTAI and then reevaluated 30 days after the second FTAI. The pregnancy at the first FTAI was calculated by the proportion of pregnant females 30 days after the first FTAI, divided by the total number of cows in the experiment and the overall pregnancy was calculated by the proportion of pregnant females 60 days after the second FTAI, divided by the total number of cows in the experiment. The service period was calculated as the number of days it took the animals to become pregnant.

2.3. Body Composition

Seven days before the expected calving date, body composition was measured using ultrasound to measure the loin eye area (LEA), thickness of subcutaneous rib fat (TSR) and thickness of subcutaneous fat on the croup (TSC). The TSC images were taken between the ileum and ischium in a rectilinear position between the two tuberosities until the identification of the upper border of the Biceps femoris, while the TSR and LEA images were obtained in the intercostal region, between the 12th and 13th ribs. The measurement of TSR (mm) and TSC (mm) / LEA (cm²) comprised the lower limit of the dermis and the deep fascia lata of the Biceps femoris and Longissimus dorsi muscles, respectively. The equipment used was the

Aloka ultrasound (SSD 500V®, Aloka, Ltd., Tokyo, Japan) with an 18-cm linear probe. The images were analyzed with BioSoft Toolbox® II for beef (Biotronics Inc., Ames, Iowa, USA).

2.4. Forage sampling

Pasture samples were collected every 30 days by handpicking (simulated grazing), to assess forage quality.

To evaluate forage availability, forage was collected by cutting 5 cm from the ground at five random points in each paddock, using a metal square of dimensions (0.5 x 0.5m) every 30 days. The evaluation of forage quality was performed by simulating manual grazing. Subsequently, all samples were weighed, dried in an oven (55°C) and then ground in a Willey mill (model 3, Arthur H. Thomas, Philadelphia, USA) at 2 and 1 mm for further analysis.

Forage and supplement samples processed on a 1 mm sieve were analyzed following the description of standard analytical procedures of the National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al, 2021), for content of DM (INCT-CA method G-003/1), crude protein (CP; INCT-CA method N-001/1), mineral matter (MM; INCT-CA method M-001/1), ether extract (EE; INCT-CA method G-004/1), neutral detergent fiber, using thermostable alpha amylase without the addition of sodium sulfite and corrected for ash and protein (aNDF; INCT-CA method F- 002/1). For quantification of indigestible neutral detergent fiber (iNDF; INCT-CA method F-009/1), an incubation procedure was performed *in situ* nonwoven textile bags (100g/m²) for 288 hours of the samples processed at 2 mm.

2.5. Milk sampling

To estimate milk production the cows were milked 30 days postpartum. In order to drain the udders of the cows, the calves were separated from their mothers from 3:00 PM to 5:45 PM when they were reunited with their mothers so that they could suckle and then drain the milk from their udders. At 6:00 pm, the calves were separated from the cows again until the next morning, during which time they were housed in a pen with access to water. The cows were milked at 5:00 am of the following day immediately after the application of 2 mL of Oxytocin (10 IU/mL, Ocitopeç®, Biovet, São Paulo, Brazil) in the mammary vein. The milk was weighed after complete extraction and about 30 mL of milk were separated from each cow to assess milk composition. The milk was weighed after complete extraction and about 30 mL of milk was separated from each cow to assess milk composition. The exact order and time that each cow was milked was recorded and then the calves were kept away

from their mothers and a new milking was performed at 6:00 pm, to obtain 24 h milk production.

The collected milk was analyzed for protein, fat, lactose and total solids content by infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil).

2.6. Blood samples

For evaluation of the metabolic and hormonal profile, blood samples were collected at 7:00 am on days -21, 0, 21 and 42 from calving (taking parturition as day 0) and for progesterone blood samples were collected at 42 days postpartum by jugular vein puncture, using sterile vacuum tubes and coagulation accelerator gel (SST II Advance®, BD Vacutainer, São Paulo, Brazil), to quantify the concentrations of urea, total proteins, albumin, triglycerides, total cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (β HB) and progesterone. A tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantify the plasma glucose concentration. After collection, samples were centrifuged at $3600 \times g$ for 20 min. Serum and plasma were immediately frozen at -20°C until analyzed.

Blood samples were analyzed using Bioclin® (Belo Horizonte, Brazil) kits to dose urea (K056), total protein (K031), albumin (K031), triglycerides (K117), total cholesterol (K083) and glucose (K082). NEFA and β HB were analyzed using Randox® kits (FA115 and RB1007, Antrim, UK). All analyses mentioned above were determined by an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). Progesterone was analyzed by Beckman kit (33,550 Beckman Coulter®, Brea, USA). Globulins were calculated by the difference between total protein and albumin. Blood urea nitrogen (NUS) was estimated as 46.67% of total serum urea.

2.7. Statistical Analysis

The experiment was conducted and analyzed in an entirely randomized experimental design with double error structure. The results were analyzed adopting the initial body weight as covariate. The analyses of variance (ANOVA) for the variables studied were performed according to the following mathematical model:

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

Where, Y_{ijk} : observation taken on individual k in paddock j subjected to treatment i ; μ : overall mean; T_i : fixed effect of treatment; $e_{(i)j}$: random, unobservable error associated with each paddock j subjected to treatment i , NID assumption $(0, \sigma e^2)$; and $\varepsilon_{(ij)k}$: unobservable

random error associated with each observation k allocated to paddock j and subjected to treatment i , NID assumption $(0, \sigma^2_\epsilon)$.

The effect of supplementation, and the linear and quadratic effects of supplementation level were evaluated by decomposition of the sum of squares through orthogonal contrasts (Steel et al., 1997). Body weight, body composition, and blood metabolites measurements were analyzed using the repeated measures procedure, where day of collection was considered the repeated variable. The most appropriate covariance structure was chosen based on the lowest value of the corrected Akaike information criterion. For variables that showed a linear or quadratic effect, a Dunnett test was performed to identify whether a supplemented treatment differed from the control. The pregnancy rate was evaluated using a chi-square test. For all statistical procedures, the PROC MIXED procedure of SAS (Statistical Analysis System; version 9.4) was used, adopting $\alpha = 0.05$ as the critical level for probability of occurrence of type I error.

3. RESULTS

The average availability of DM and pdDM during the experimental period was 4087 and 2970 kg/ha, respectively. For the forage samples collected via simulated manual grazing, the average CP content was 77.7 g/kg DM (Table 2).

The BW of cows showed positive linear behavior in the prepartum with supplementation plan and presented differences in relation to the day of weighing ($P < 0.001$; Figure 2), and the animals that received 4 and 6 g/kg BW supplement presented differences in relation to the animals of the control treatment with higher body weight gain 7 days before calving and for these treatments the animals presented a lower loss of BW at 90 days postpartum ($P < 0.05$; Table 3; Figure 2), differing from the animals of the control treatment. Animals in the 2 g/kg BW treatment did not differ from the control treatment for BW variation at pre and postpartum ($P > 0.05$; Table 3; Figure 2).

The results for BCS at birth and at 90 days postpartum presented a positive linear effect with increasing supplementation ($P < 0.05$; Table 3), and the variation in BCS presented a positive linear effect in the prepartum ($P < 0.05$; Table 3), being higher for animals in the treatments 4 and 6 g/kg BW, however, there was no difference in the postpartum ($P > 0.05$; Table 3). For the measurements of LEA, TSR and TSC there was a positive linear effect as the supply of supplement increased in the prepartum (Table 3; $P < 0.05$).

Regarding calf performance, no effect of the supplementation plan was observed on birth weight and at 90 days of age ($P > 0.05$; Table 3). There were no differences for overall

pregnancy ($P>0.05$; Table 4), and for pregnancy at first FTAI there was a tendency for greater pregnancy as the supplement increased ($P=0.07$; Table 4), however, a positive linear effect was observed for the service period ($P<0.05$; Table 3), in which a lower service period was observed for animals in the 4 and 6 g/kg CP treatments compared to animals in the control treatment ($P<0.05$; Table 3), and animals in the 2 g/kg CP treatment did not differ from the control ($P>0.05$; Table 3). Milk yield and composition were not influenced by treatments ($P>0.05$; Table 5).

For the concentrations of BUN, β HB and NEFA there was an interaction of treatment with the day of blood collection ($P<0.05$; Table 5), for the other metabolites there were differences only regarding the day of collection ($P<0.01$; Table 6).

For glucose (Figure 3), higher concentrations were observed at parturition (73.5 mg/L), at 21 days postpartum the concentrations reduced and remained stable ($P<0.05$). Lower serum concentrations were observed for total cholesterol ($P<0.05$; Figure 4) at parturition and at 21 days postpartum, which then increased from day 21 to day 42.

BUN concentrations were highest for cows receiving 6 g/kg BW on day -21, with these concentrations reducing until 21 days postpartum and then stabilizing ($P<0.05$; Figure 5). Triglyceride concentrations reduced from day -21 until calving, holding constant over time ($P<0.05$; Figure 6).

Total protein concentrations decreased at parturition, being equal at 21 days postpartum ($P>0.05$; Figure 7A) and thereafter increased with the days until day 42 ($P<0.05$; Figure 7A). Albumin decreased until day 21 postpartum and subsequently increased ($P<0.05$; Figure 7B), whereas globulins decreased only at parturition ($P<0.05$; Figure 7C), and maintained similar concentrations on days -21, 21, and 42 postpartum ($P>0.05$; Figure 7C).

The concentration of β HB, was higher in the prepartum for animals in the control treatment ($P<0.05$; Figure 8), while for supplemented animals presented the behavior of increasing with time ($P<0.05$; Figure 8). The NEFA concentrations were lower 21 days before parturition for the animals of the treatments that received 4 and 6 g/kg BW and at parturition the concentrations were high and without differences in the treatments and at 21 and 42 days after parturition were lower for the treatments that received 4 and 6 g/kg BW ($P<0.05$; Figure 9), in all treatments, the same behavior of reduction of NEFA concentrations with time was observed after parturition (Figure 9).

Progesterone concentrations showed a positive linear effect with increasing supplement supply ($P<0.05$; Table 6), and higher progesterone concentration was observed at 42 days for animals receiving 4 and 6 g/kg BW compared to animals in the control treatment

($P < 0.05$; Table 6), animals in the 2 g/kg BW treatment did not differ from the control ($P > 0.05$; Table 6).

4. DISCUSSION

In this study the availability of forage was not a limiting factor for animal performance, considering the supply of pdDM of 55 g/kg of animal BW during the entire experimental period, higher than the range suggested by Paulino et al. (2004) of 40 to 50 g/kg BW. This justifies the good performance of the animals in the control treatment and the animals that received 2 g/kg BW.

During the last 60 days of pregnancy, cows have higher nutritional needs (Valadares Filho et al., 2016) and, according to da Silva et al. (2017), supplementation administered in adequate amounts during this period can have beneficial effects on energy and protein metabolism of cows, not only due to the increase in body reserves, but also due to its effects on cow metabolism later when supplements are no longer provided. These metabolism results found, provide evidence that adopting higher levels of supplementation in the prepartum period improves postpartum cow performance.

The levels of supplementation in the prepartum, influenced the BW at 45 days postpartum, and the animals that presented the greatest variation in BW in the prepartum, treatments 4 and 6 g/kg BW, obtained less loss of BW at 90 days postpartum, indicating a lower negative energy balance (NEB), evidenced by the lower concentration of NEFA. Da Silva et al. (2017), under similar conditions of the present study, also observed lower weight loss in animals that received 1.5 kg of supplement (3 g/kg BW) for 60 days prepartum.

Previous studies in cattle during pregnancy (Radunz et al., 2010; Radunz et al., 2011) have provided evidence that supplementation systems during the last third of pregnancy can alter calf birth weight, suggesting that the source of energy and protein may affect fetal growth, which could lead to problems with dystocia. Calf birth weight is a major factor in increasing the risk of dystocia (Boakari & Ali, 2021) and in such cases calves typically perform inferiorly compared to animals that have had non-dystocia births (Boakari & Ali, 2021). Furthermore, dystocia usually leads to delayed return to estrus by cows postpartum (Patterson et al., 1987).

However, in this study there were no differences in BW of calves at birth and no cases of dystocia. And the performance of calves presented no differences at 90 days postpartum. Corroborating the results of this study, Summers et al. (2015) and da Silva et al. (2017), found no difference in calf BW at birth according to the supplementation applied to the cows.

According to some studies (Hess et al., 2005; Marques et al., 2016), BCS is a determining factor for cows to return to early estrus with better conception rates. Furthermore, for cows with adequate BCS in the final third of pregnancy, there is evidence that body reserves can be utilized during pregnancy without compromising subsequent reproductive function (Diskin & Kenny, 2016).

Data in the literature, reveal that restricted intake in pregnant cows during the late pregnancy period results in weight loss, loss of BCS and elevated serum concentrations of NEFA and β HB, which leads to long periods of NEB in the postpartum period in both dairy (Barber et al. 1997; Bauman et al. 2000) and beef cows (Mulliniks et al., 2013).

According to Wood et al. (2013), non-supplemented pregnant cows can metabolize energy reserves and alter their metabolism to meet the energy requirements of the growing fetus without altering intake or overall growth, an example of these mechanisms is an increase in circulating β HB accompanied by a reduction in body fat. As observed in this study, β HB concentrations differed between treatments at prepartum with higher concentrations for the non-supplemented animals and lower fat thickness in the rib and croup compared to the other supplemented treatments.

Elevated β HB concentrations are indicative of a lack of energy and nutrients in the animal body, leading to mobilization of its body reserves, resulting from poor adaptation to NEB (Herdt, 2000). In beef cows, reduced serum β HB concentration prior to breeding was associated with increased pregnancy rates at first service (Mulliniks et al., 2013).

Although there was a difference between treatments in β HB concentrations on day -21, these levels do not indicate a strong mobilization of body reserve for the non-supplemented animals as observed by Mulliniks et al, (2013) who found values greater than 0.71 mmol/L of β HB at 30 days prepartum, but rather that the cows had different nutrient balances during prepartum as also observed by Lana Ferreira et al. (2020), in which it was evident for animals in the control and 2g/kg BW treatment, which also presented greater weight loss postpartum.

The interval between calving and conception greatly influences the profitability of beef production. Thus, in beef cattle systems, it is important that the cow is pregnant within 85 days of calving to ensure that the cow produces one calf per year (da Silva et al., 2017). Although no effect was observed on the overall pregnancy rate of cows, it is observed that animals that received 4 or 6 g/kg BW of supplement had a higher number of pregnant animals at the first FTAI. In addition, they had a shorter service period (Table 3), which can impact

future calf performance, as calves born earlier in the calving season have higher BW at weaning (Funston et al., 2012).

The fact that cows that received 4 and 6 g/kg BW, lost less weight from calving to 45 days postpartum in relation to control treatment cows evidences that they were less affected with NEB. Furthermore, postpartum NEFA concentrations reduced with time in relation to calving, suggesting recovery of the nutritional status of the animals, also observed by the variation in BW of the animals, but for the animals that received 4 and 6 g/kg BW supplement, these concentrations at postpartum were lower than the control treatment. Thus, these differences in blood concentrations between treatments reinforce that supplementation with 4 and 6 g/kg BW during the prepartum period influenced the energy requirements and the intensity of postpartum catabolism of cows, resulting in higher NEB for non-supplemented animals and animals of the 2 g/kg BW treatment, as previously discussed which having a higher BW. In fact, the animals in the treatments 4 and 6 g/kg BW, obtained higher levels of progesterone at 42 days after calving in relation to the animals in the control treatment, which resulted in lower NEB and consequently reducing the BW of these animals and shorter period of service.

Total cholesterol concentrations reduced at calving and increased progressively with days for all nutritional plans. Corroborating with Ruas et al. (2000) and Godoy et al. (2004) for postpartum blood cholesterol in lactating beef cows and the reduction in triglyceride concentrations postpartum suggests their use as energy demand for lactation, as they are important sources of long chain fatty acids for milk fat synthesis (Aeberhard et al., 2001).

Albumin concentrations decreased significantly at parturition until 21 days after calving, which could be related to the higher requirement of amino acids for milk production (Contreras, 2000). At calving, globulins presented lower levels compared to the rest of the period, which is justified by the transfer of immunity to colostrum production (Weaver et al. 2000), which is also reflected in the behavior of total protein concentrations.

In view of the results found in the present study, all nutritional plans presented acceptable calving interval, however, the animals that received 4 and 6 g/kg BW supplementation presented a service period. Thus, with the increase in earlier calving due to earlier pregnancy can increase the value of calves at weaning, thus justifying a higher supplementation level, which in this case, does not differ between the use of 4 and 6 g/kg BW in supplement, i.e., economically, the use of 4 g/kg BW is the most indicated.

5. CONCLUSION

Providing 4g/kg BW of protein-energy supplement to grazing Nellore cows 60 days prior to calving is recommended, which improves metabolic characteristics and performance in the prepartum and postpartum period and a lower negative energy balance in the postpartum period, resulting in a shorter service period.

6. REFERENCES

- Aeberhard.K; Bruckmaier, R.M; Blum, J.W. Metabolic, enzymatic and endocrine status in high yielding dairy cows – part 2. *Journal of Veterinary Medicine Series*, v. 8, p.111-127, 2001.
- Astessiano, A.L; Pérez-Clariget; Espasandí, A.C. et al. Metabolic, productive and reproductive responses to postpartum short-term supplementation in primiparous beef cows. *Revista Brasileira de Zootecnia*, v. 42, p. 246-253, 2013.
- Ayres, H.; Ferreira, R. M.; Torres-Júnior, J. R. S.; Demétrio et al. Inferences of body energy reserves on conception rate of suckled Zebu beef cows subjected to timed artificial insemination followed by natural mating. *Theriogenology*, v. 82, n. 4, p.529-536, 2014.
- Barber, M.C; Clegg, R.A; Travers, M.T et al. Lipid metabolism in the lactating mammary gland. *Biochim Biophys Acta*, v. , n. p.101-126. 1997.
- Baruselli, P.S; Reis, E.L; Marques, M.O et al. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Animal Reproduction Science*, v. 82, p. 479-486, 2004.
- Bauman, D.E. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. In: *Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction*. Proceedings of the 9th International Symposium on Ruminant Physiology. Edited by Cronje PB. New York: CABI Publishing. Edited by Cronje PB. New York: CABI Publishing. p.311. 2000.
- Bell, A; Ehrhardt, R. Regulation of macronutrient partitioning between maternal and conceptus tissues in the pregnant ruminant. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*; CABI International: Walling-ford (OX), UK, Cronjé, P.B. p.275-293. 2000.
- Boakari, Y.L. and Ali, H.E.-S. (2021). Management to Prevent Dystocia. In *Bovine Reproduction*, R.M. Hopper (Ed.). <https://doi.org/10.1002/9781119602484.ch49>

- Cardenas, J.E.G. Nutritional and metabolic evaluation of Nellore cows supplemented or not during the peripartum. PhD Thesis – Federal University of Viçosa. Animal Science Department. Animal Science Department. 2017.
- Contreras, P. Indicators of protein metabolism used in herd metabolic profiles. In: FHD G, Barcellos JO, Ospina H, LAO R, editors. In: Metabolic profiling in ruminants: its use in nutrition and nutritional diseases, Porto Alegre: Federal University of Rio Grande do Sul Printing Office; v1, 2000.
- De Almeida, D. M; Marcondes, M. I; Rennó, L. N; Martins, L. S; Contreras, D; Saldarriaga, F. V; Villadiego, F. A. C; Ortega, R. M; Moreno, D. P. S; Moura, F. H; Cunha, C. S; Paulino, M. F. Effects of pre- and postpartum supplementation on lactational and reproductive performance of grazing Nellore beef cows. *Animal Production Science* , v. 61, p. 101-107, 2020.
- Detmann, E; Costa e Silva, L.F; Palma, M.N.N. et al. Methods for food analysis – INCT – Ciência Animal. 2ª edition. Visconde do Rio Branco. Suprema: Brazil. p.350. 2021.
- Detmann, E; Valadares Filho, S.C; Paulino et al. Nutritional aspects applied to grazing cattle in tropics: a review based on Brazilian results. *Semina: Ciências Agrárias*, v. 35, n. 4, p.2829-54, 2014.
- Diskin, M.G; Kenny, D.A. Managing the reproductive performance of beef cows. *Theriogenology*, v. 86, p. 379-87, 2016.
- Funston, R.N; Musgrave. J.A; Meyer, T.L; Larson, D.M. Effect of calving distribution on beef cattle progeny performance. *J Anim Sci*. v.90. p.5118–21, 2012.
- Foster, L.A. Clinical ketosis. *Veterinary Clinics of North America: Food Animal Practice*, v. 4, n. 2, p.253-267, 1998.
- Godoy, M.M; Alves, J.B; Monteiro, A.L.G. et al. Reproductive and metabolic parameters of Guzerá cows supplemented in pre and postpartum. *Revista Brasileira de Zootecnia*, v. 33, p.103-111, 2004.
- Herdt, T.H. Ruminant adaptation to negative, energy balance. Influences on the etiology of ketosis and fatty liver. *Veterinary Clinics of North America: Food Animal Practice*, v. 16, n.2, p. 215-230, 2000.
- Hess, B.W; Lake, S.L; Scholljegerdes, E.J. Nutritional controls of beef cow reproduction. *Journal of Animal Science*, v. 83, n.13, p. 90-106, 2005.
- Holtenius, K; Agenas, S; Delavaud, C . et al. Effect of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *Journal of Animal Science*, v. 86, n. 3, p. 883-891, 2003.

- Kolnes, A.J; Birk, J.B; Eilertsen, E. et al. Epinephrine-stimulated glycogen breakdown activates glycogen synthase and increases insulin-stimulated glucose uptake in epitrochlearis muscles. *American journal of physiology-endocrinology and metabolism*, v. 308, n. 3, p. 231-240, 2015.
- Lana Ferreira, M. F; Rennó, L. N.; Detmann, E. et al. Performance, metabolic and hormonal responses of grazing Nellore cows to an energy-protein supplementation during the pre-partum phase. *BMC Veterinary Research*, v. 16, n.1, p.1-13. 2020.
- Lazzarini, I; Detmann, E; Sampaio, C. B. et al. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia*, v. 38, p. 2021-2030, 2009.
- Lopes, S.A.; Paulino, M.F; Detmann, E. et al. Evaluation of grazing beef cows receiving supplements with different protein contents. *Semina: Ciências Agrárias*, v. 37, n. , p.3361-3372, 2016.
- Marques, R.S; Cooke, R.F; Rodrigues, M.C. et al. Impacts of cow body condition score during gestation on weaning performance of the offspring. *Livestock Science*, v. 191, p.174-178, 2016.
- Mulliniks, J. T; Kemp, M. E; Endecott, R. L. et al. Does β -hydroxybutyrate concentration influence conception date in young postpartum range beef cows. *Journal of Animal Science*, v. 91, n. , p.2902-2909, 2013.
- National Research Council – NRC. Nutrient requirements of beef cattle. Washington: Academic Press. 2001.
- National Research Council – NRC. Nutrient requirements of beef cattle. Washington: Academic Press. 2016.
- Paulino, M.F; Detmann, E; Valadares Filho, S.C. et al. Programmed Cattle Cultivation. In: *Annals of the VII Symposium on Cattle Production*. Viçosa. p.267-297. 2010.
- Paulino, M.F; Detmann, E; Valente, E.E.L. et al. Nutrition of cattle on pasture. In: *Symposium on Strategic Grazing Management*. Viçosa. p.131-169. 2008.
- Patterson, D., Bellows, R., Burfening, P., and Carr, J. (1987). Occurrence of neonatal and postnatal mortality in range beef cattle. I. Calf loss incidence from birth to weaning, backward and breech presentations and effects of calf loss on subsequent pregnancy rate of dams. *Theriogenology* 28: 557–571.
- Payne, J.M; Payne, S. The metabolic profile test. Oxford: Oxford University, in press; 1987.
- Radunz, A. E; Fluharty, F. L; Day, M. L. et al. Prepartum dietary energy source fed to beef cows: I.Effects on pre- and postpartum cow performance. *Journal of Animal Science*.

- v. 88, n. , p.2717-2728, 2010.
- Radunz, A.E; Fluharty, F.L; Zerby, H.N. et al. Winter-feeding systems for gestating sheep I. Effects on pre- and postpartum ewe performance and lamb progeny preweaning performance. *Journal of Animal Science*, v. 89, n. , p. 467-477, 2011.
- Reist, M; Koller, A; Busato, A. et al. First ovulation and ketone body status in the early postpartum period of dairy cows. *Theriogenology*, v. 54, p. 685-701, 2000.
- Ruas, J.R.M; Torres, C.A.A; Valadares Filho, S.B.C. et al. Effect of protein supplementation to pasture on forage intake, weight gain and body condition, in Nelore cows. *Revista Brasileira de Zootecnia*, v. 29, p.930-934, 2000.
- Sampaio, C. B; Detmann, E; Lazzarini, I. et al. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia*, v. 38, p.560-569, 2009.
- Santos, J. E. P; Cerri, R. L. A; Sartori, R. Nutritional management of the donor cow. *Theriogenology*, v. 69, n.1, p.88-97, 2008.
- Sartori, R.; Guardieiro, M. M. Nutritional factors associated with reproduction in the female bovine. *Revista Brasileira de Zootecnia*, v. 39, n. SUPPL. 1, p. 422–432, 2010.
- Silva, A.G; Paulino, M.F; Detmann, E. et al. Energetic-protein supplementation in the last 60 days of gestation improves performance of beef cows grazing tropical pastures. *Journal of Animal Science and Biotechnology*, v. 8, p.78-87, 2017.
- Sotelo, D; Paulino, M. F; Rennó, L. N. et al. Performance and metabolic status of grazing beef heifers receiving increasing protein supplementation pre- and postpartum. *Animal Production Science*, v. 59, p.1244-1252, 2018.
- Summers, A.F; Meyer, T.L; Funston, R.N. Impact of supplemental protein source offered to primiparous heifers during gestation on I. Average daily gain, feed intake, calf birth body weight, and rebreeding in pregnant beef heifers. *Journal of Animal Science*. v. 93, p.1865-1870, 2015.
- Valadares Filho, S.C; Costa e Silva, L.F; Gionbelli, M.P. et al. BR-CORTE 3.0 - Nutrient requirements of zebu and crossbred cattle. 3rd Ed. Viçosa: Federal University of Viçosa. 2016.
- Walsh, R. B; Walton, J. S; Kelton, D. F. et al. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. *Journal of dairy science*. v90, p.2788–2796., 2007.
- Weaver, D. M; Tyler, J. W; VanMetre, D. C. et al. Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine*, v. 14, p. 569-

577, 2000.

Wiltbank, M; Lopez, H; Sartori, R. et al. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*, v. 65, n.1, p. 17-29, 2006.

Wood, K.M; Awda, B.J; Fitzsimmons, C. et al. Influence of pregnancy in mid-to-late gestation on circulating metabolites, visceral organ mass, and abundance of proteins relating to energy metabolism in mature beef cows. *Journal of Animal Science*, v. 91, p. 5775-5784, 2013.

7. TABLES

Table 1: Supplement composition (g/kg) as feed

Item	Supplement
Ingredients (g/kg)	
Ground corn	258
Wheat bran	258
Soybean meal	479
Urea:ammonium sulfate (9:1)	5
mineral mixture composition: dicalcium phosphate (500.0 g/kg), sodium chloride (471.9 g/kg), zinc sulfate (15.0 g/kg), copper sulfate (7.0 g/kg), cobalt sulfate (0.5 g/kg), potassium iodide (0.5 g/kg), sodium selenite (0.1 g/kg) and manganese sulfate (5.0 g/kg).	

Table 2: Chemical composition of the supplement and forage

Item	Supplement	Forage				
		August	September	October	November	December
DM ¹	892.3	651.2	665.4	516	234.4	238.6
OM ²	965	931.8	933.6	934.7	933.7	934.1
CP ²	308.9	54.9	51.3	62.5	100.2	102.3
apNDF ²	163.3	749.2	780.6	709.4	530.3	535.1
iNDF ²	56.7	339.6	352.1	330.8	166.9	170.5

DM – Dry matter, OM – Organic matter, CP – Crude protein, apNDF - neutral detergent fiber corrected for ash and protein, iNDF - indigestible neutral detergent fiber

¹ g/kg of natural material

² g/kg of DM

Table 3: Effect of supplementation levels on cow and calf performance

Item ¹	Treatments (g/kg BW)				SEM	P-value ²		
	Control	2	4	6		C x S	L	Q
Cows								
Initial BW, kg	508	524	524	522	16.62	0.465	0.599	0.607
Calving BW, kg	494	505	525	538	16.79	0.205	0.11	0.956
Variation BW prepartum, kg	22.6	22.3	44.2	62.7*	6.577	0.053	0.008	0.209
BW variation 45 days Postpartum, kg	-22.9	-19.6	-9.9	-8.9	5.478	0.183	0.839	0.101
BW variation 90 days Postpartum, kg	-7.1	-5.5	0.0*	2.7 *	2.428	0.091	0.032	0.815
Initial BCS	5.9	6.0	6.1	6.0	0.149	0.401	0.589	0.423
Calving BCS	6.4	6.8	7.5*	7.7*	0.164	0.007	0.015	0.454
Final BCS	5.5	5.8	6.3*	6.7*	0.208	0.032	0.011	0.789
Variation of prepartum BCS	0.51	0.77	1.4*	1.65*	0.179	0.021	0.007	0.977
BCS variation 90 days Postpartum	-0.83	-0.97	-1.15	-0.89	0.126	0.296	0.575	0.169
LEA, cm ²	42.6	45.6	48.5*	49.8*	1.171	0.015	0.009	0.479
TSR, mm	2.6	3.2	4.2*	4.9*	0.238	0.004	0.001	0.854
TSC, mm	3.2	3.6	4.9*	5.4*	0.382	0.028	0.008	0.894
Serving period, days	81	76	57*	56*	5.749	0.054	0.020	0.784
Calves								
BW Birth, kg	37	36	35	37	1.754	0.744	0.878	0.396
BW 90 days, kg	87	91	89	95	3.091	0.246	0.155	0.666
ADG, kg/d	0.97	1.01	0.99	1.06	0.347	0.252	0.157	0.653

¹ BW – Body weight; BCS – Body Condition Score; LEA – Loin Eye Area; TSR – thickness of subcutaneous rib fat; TSC - thickness of subcutaneous fat on the croup ; FTAI – fixed-time artificial insemination; ADG – Average daily gain.

² L e Q - linear and quadratic order effects.

* Means statistically different from the control by Dunnett's test.

Table 4: Effect of supplementation levels on cow reproduction

Item ¹	Treatments (g/kg BW)				SEM	P-value
	Control	2	4	6		
First FTAI pregnancy, %	33.3	45.4	70	88.9	-	0.0701
Overall pregnancy, %	77.8	81.8	88.9	88.9	-	0.9386

¹ FTAI – fixed-time artificial insemination;

Table 5: Effect of supplementation levels on milk yield and composition

Item	Treatments (g/kg BW)				SEM	P-value ¹		
	Control	2	4	6		C x S	L	Q
Production, kg/d	7.5	7.8	7.6	7.7	0.439	0.698	0.825	0.831
Fat, %	5.6	5.5	5.3	5.5	0.337	0.691	0.749	0.697
Protein, %	4.5	4.5	4.5	4.4	0.129	0.859	0.711	0.726
Lactose, %	4.5	4.5	4.5	4.6	0.104	0.674	0.548	0.853
Total solids, %	15.2	15.3	15.0	15.2	0.376	0.981	0.924	0.919

¹ L e Q - linear and quadratic order effects.

* Means statistically different from the control by Dunnett's test.

Table 6: Effect of supplementation levels on the metabolic profile of cows in pre and postpartum

Item ¹	Treatments (g/kg BW)				SEM	P-value ²				
	Control	2	4	6		C x S	L	Q	Day ³	Treat x Day
Glucose, mg/dL	61.3	61.9	62.9	61.2	1.338	0.948	0.921	0.416	<.0001	0.489
BUN, mg/dL	11.6	12.9	13.7*	14.6*	0.635	0.060	0.04	0.771	<.0001	<0.001
Total Cholesterol, mg/dL	100.3	106	108	100.5	3.096	0.363	0.893	0.151	<.0001	0.995
Triglycerides, mg/dL	20.2	22.1	22.3	20.1	0.924	0.291	0.97	0.084	<.0001	0.160
Total Protein, g/dL	6.8	6.7	6.7	6.5	0.114	0.354	0.23	0.792	0.0002	0.871
Albumin, g/dL	2.9	2.9	3.0	3.0	0.051	0.282	0.15	0.697	<.0001	0.707
Globulins, g/dL	3.9	3.8	3.7	3.6	0.127	0.188	0.1	0.956	0.003	0.996
βHB, mmol/L	0.47	0.43*	0.42*	0.42*	0.011	0.015	0.02	0.105	<.0001	0.013
NEFA, mmol/L	0.25	0.23	0.20	0.18	0.017	0.033	0.01	0.782	<.0001	0.001
Progesterone, ng/mL	0.22	0.46	1.19*	1.22*	0.179	0.023	0.01	0.584	-	-

¹ BUN- Blood urea nitrogen; βHB – β-hydroxybutyrate; NEFA – Non-esterified fatty acids

² L e Q - linear and quadratic order effects.

³ Day related to the birth.

* Means statistically different from the control by Dunnett's test.

8. FIGURES

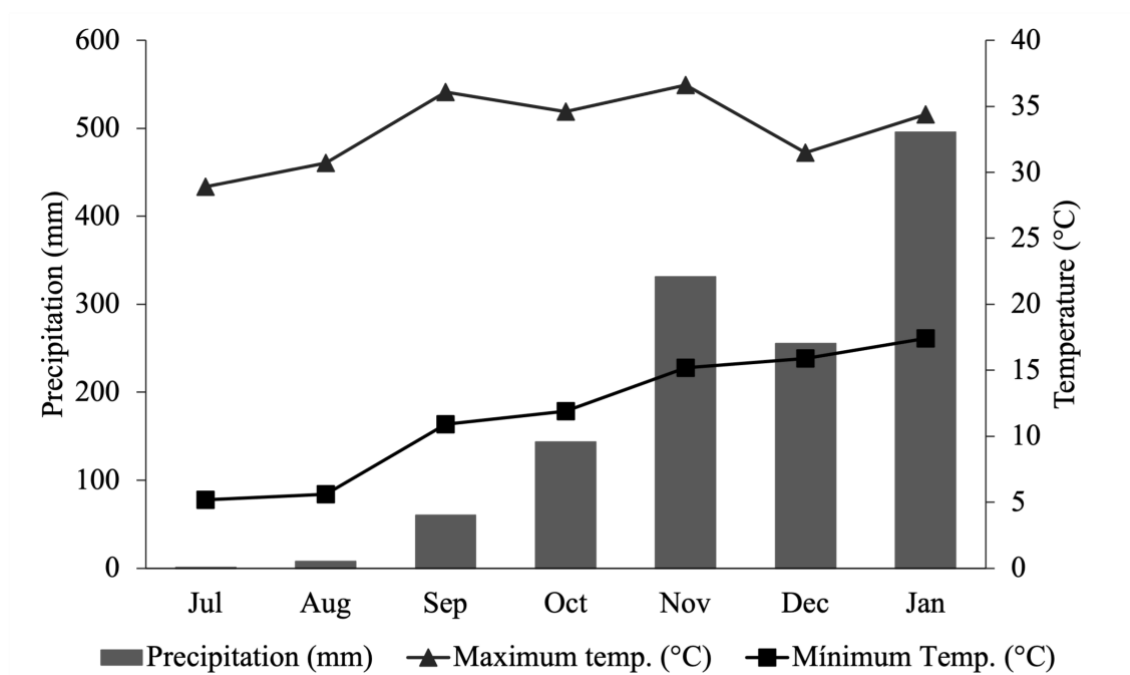


Figure 1- Precipitation and average temperature during the experimental period. Viçosa - MG.

Source: INMET

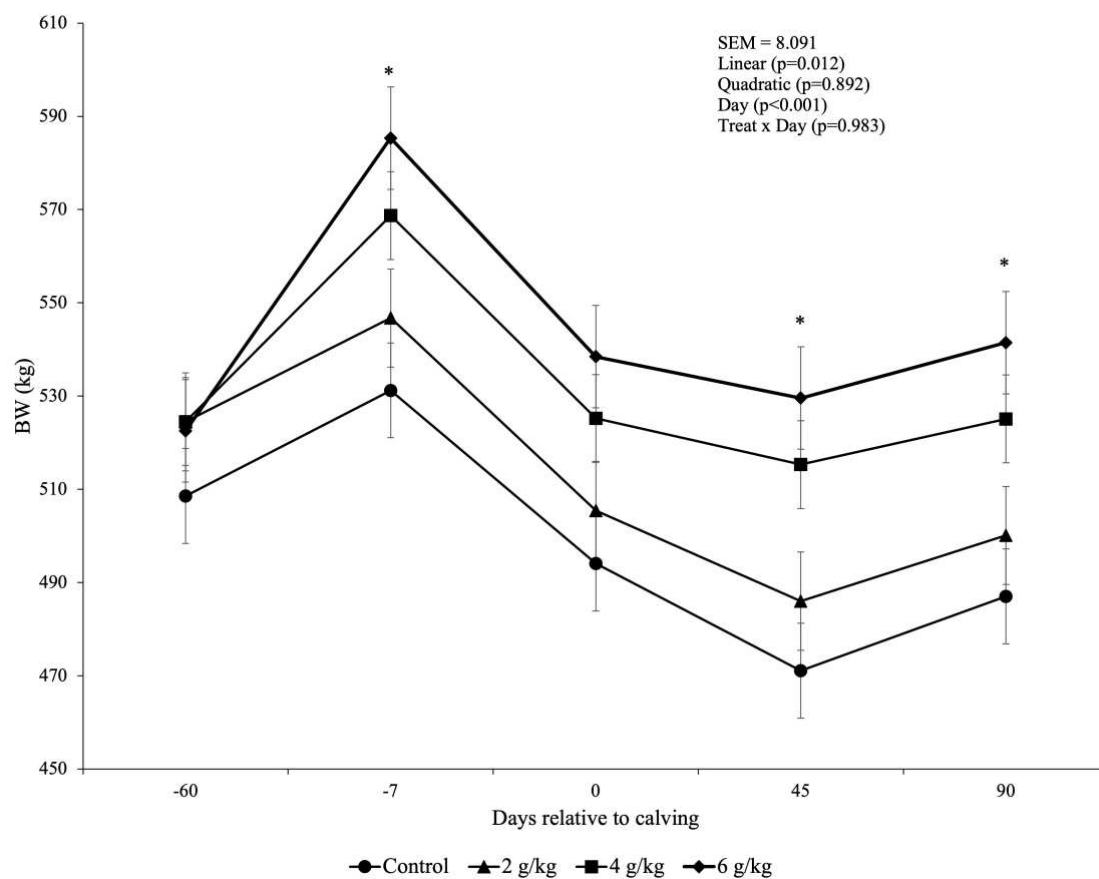


Figure 2- Body weight (BW) during the pre- and post-partum period. Asterisks (*) indicate significant differences between treatments ($P < 0.05$)

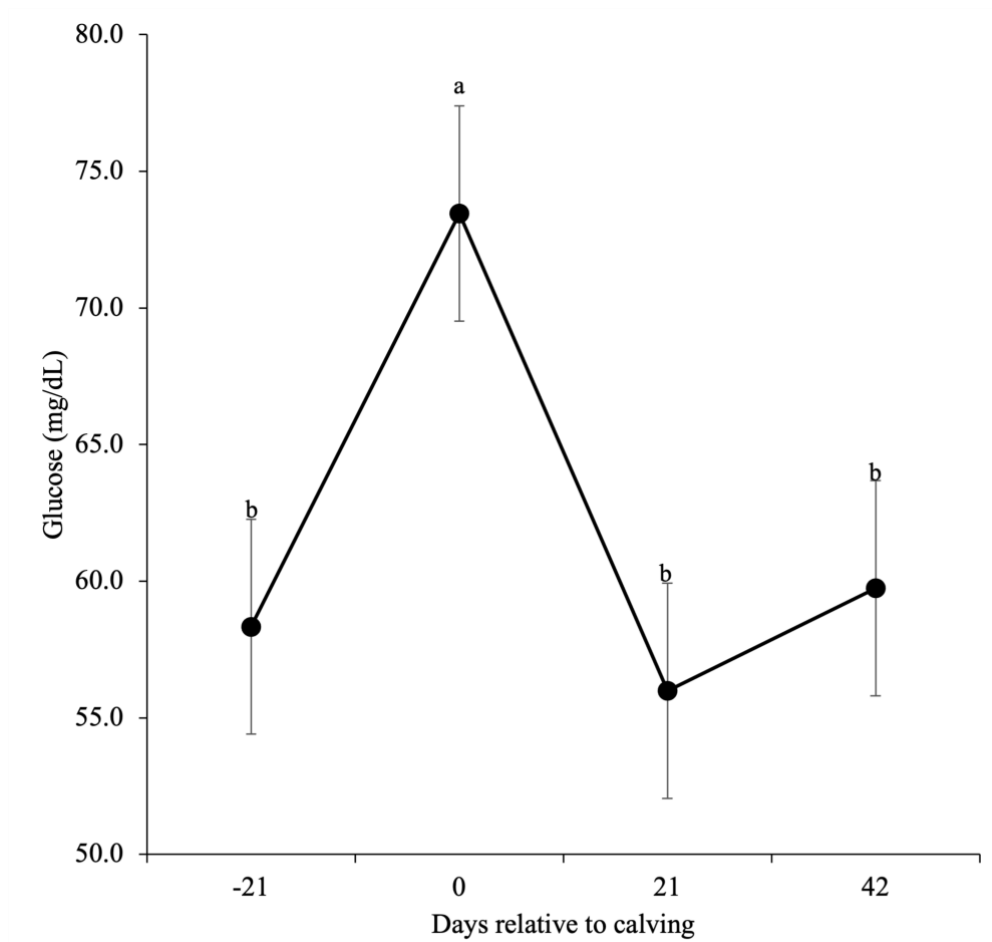


Figure 3- Glucose concentrations during pre- and post-partum. Different letters indicate significant differences between collection days ($P < 0.05$)

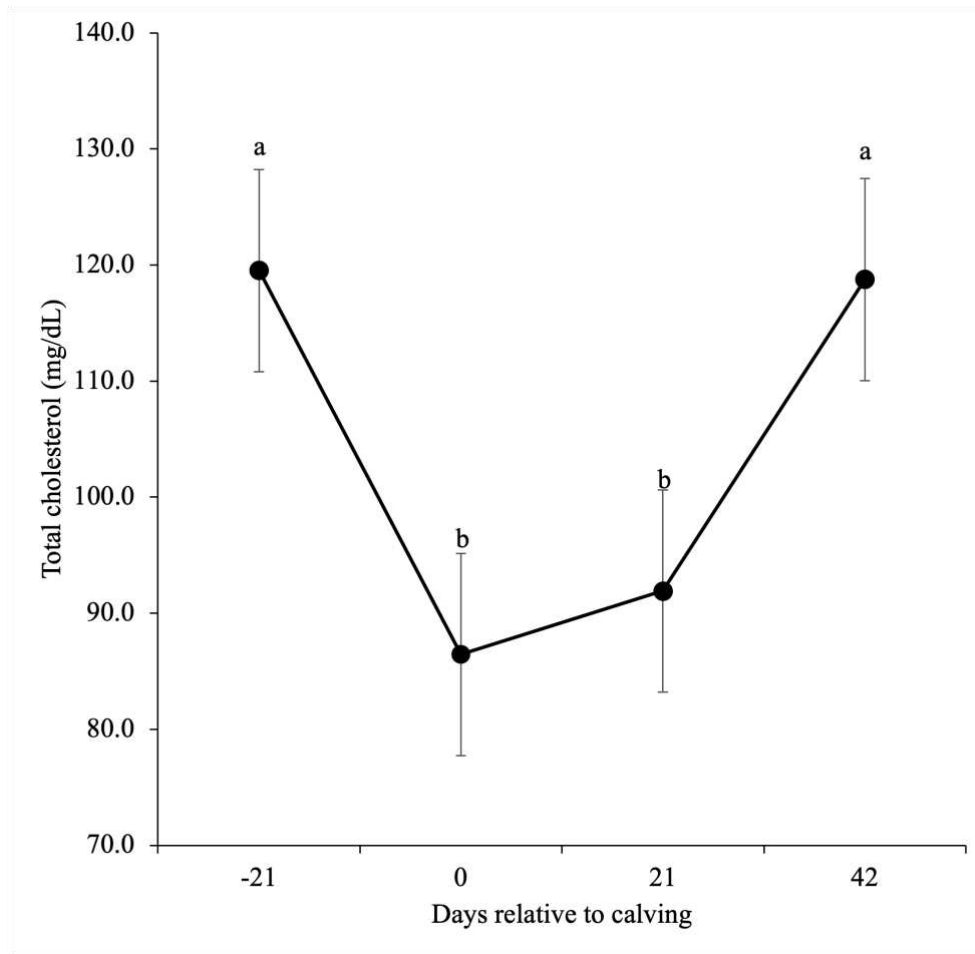


Figure 4 - Concentrations of total cholesterol during the pre- and post-partum period. Different letters indicate significant differences between collection days ($P < 0.05$)

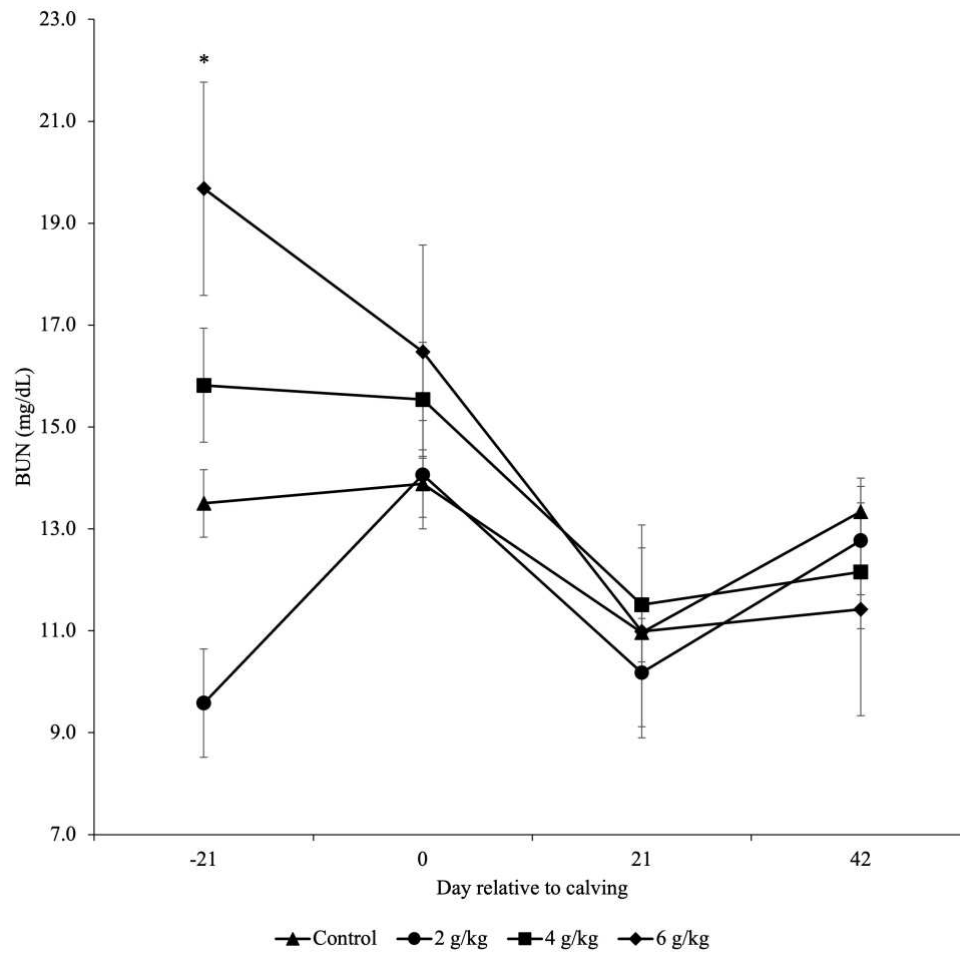


Figure 5 - Blood urea nitrogen (BUN) concentrations during pre- and postpartum. Asterisks (*) are significantly different between treatments ($P < 0.05$)

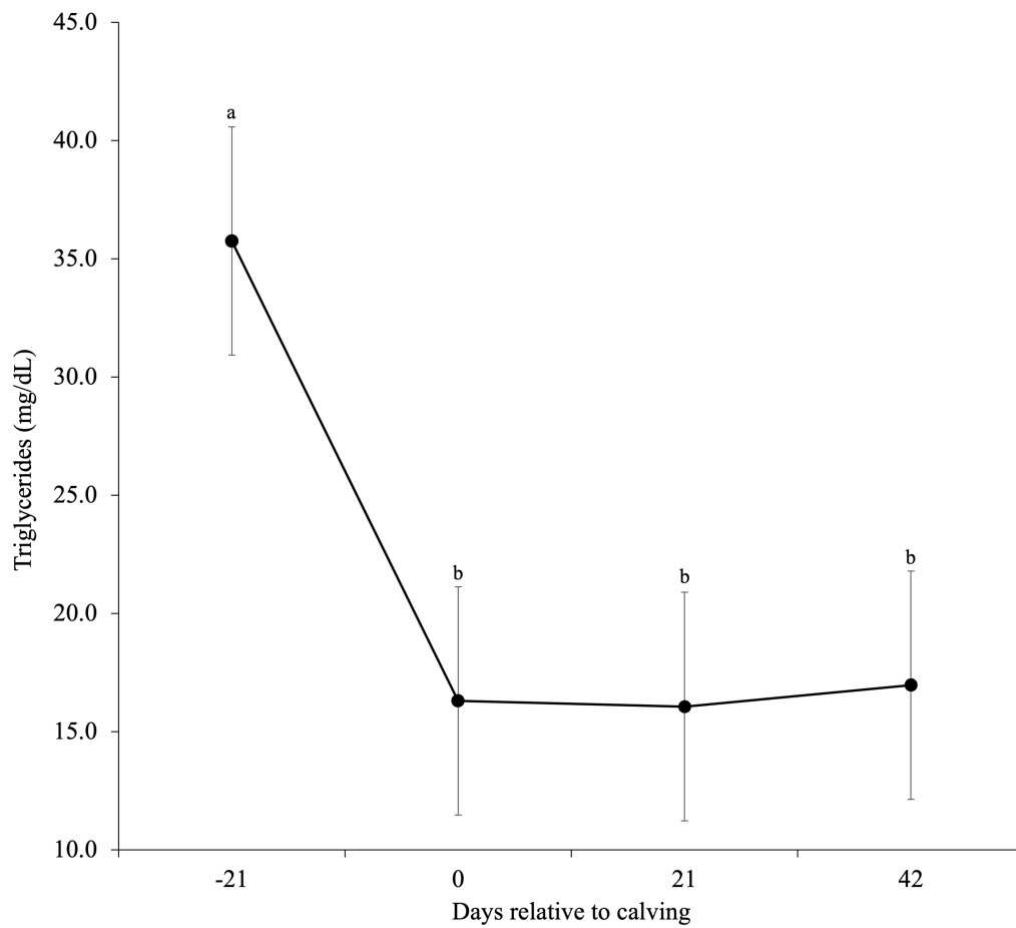
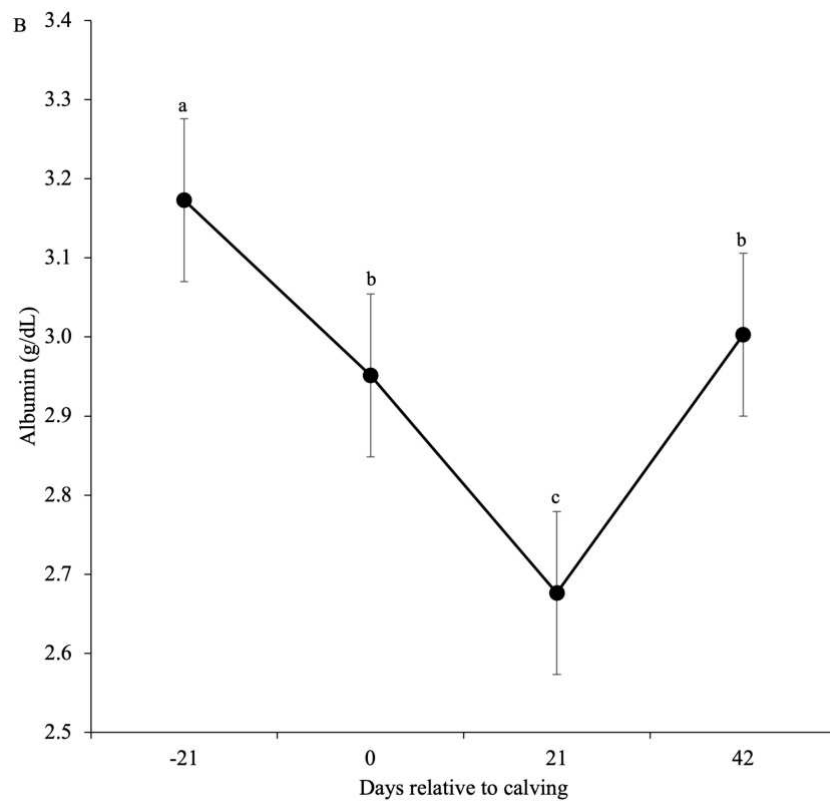
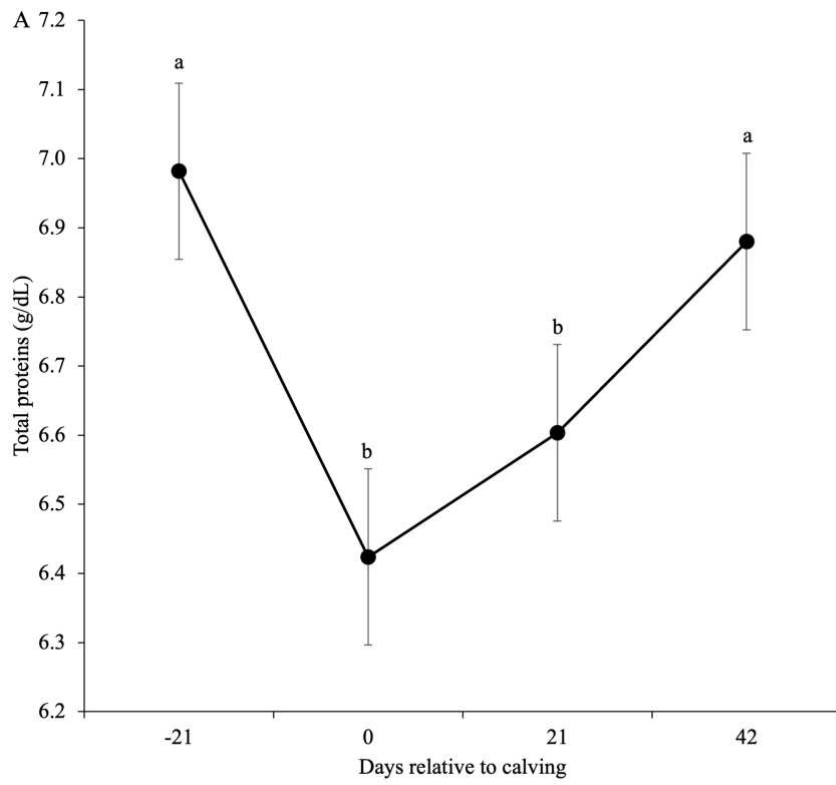


Figure 6- Triglyceride concentrations during pre- and post-partum. Different letters indicate significant differences between collection days ($P < 0.05$)



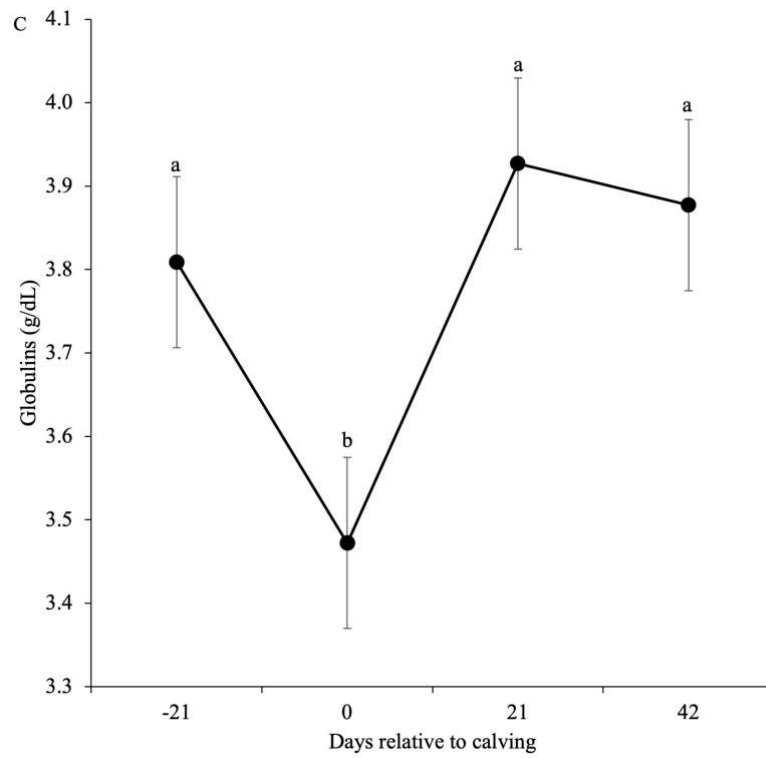


Figure 7- Concentrations of total proteins (A); albumin (B) and globulins (C) during pre and postpartum. Different letters indicate significant differences between days ($P < 0.05$)

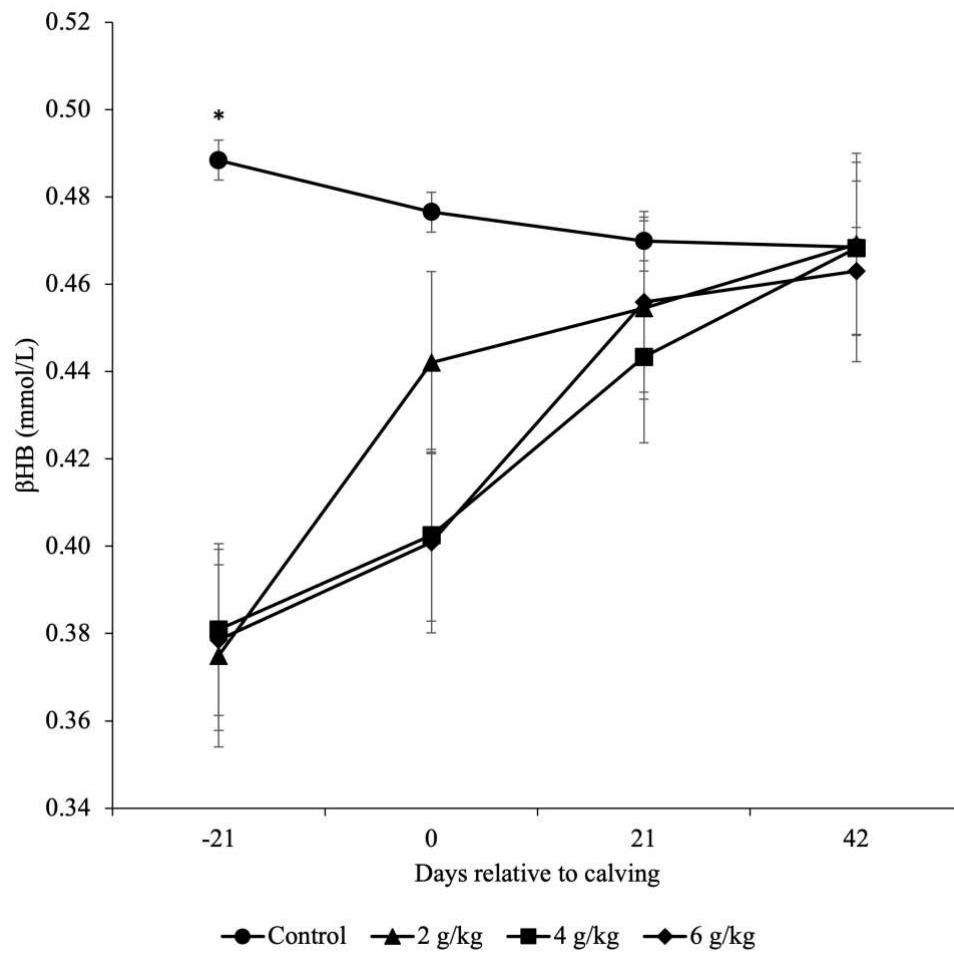


Figure 8- Concentrations of β -hydroxybutyrate (β HB) during pre- and postpartum. Asterisks (*) are significantly different between treatments ($P < 0.05$)

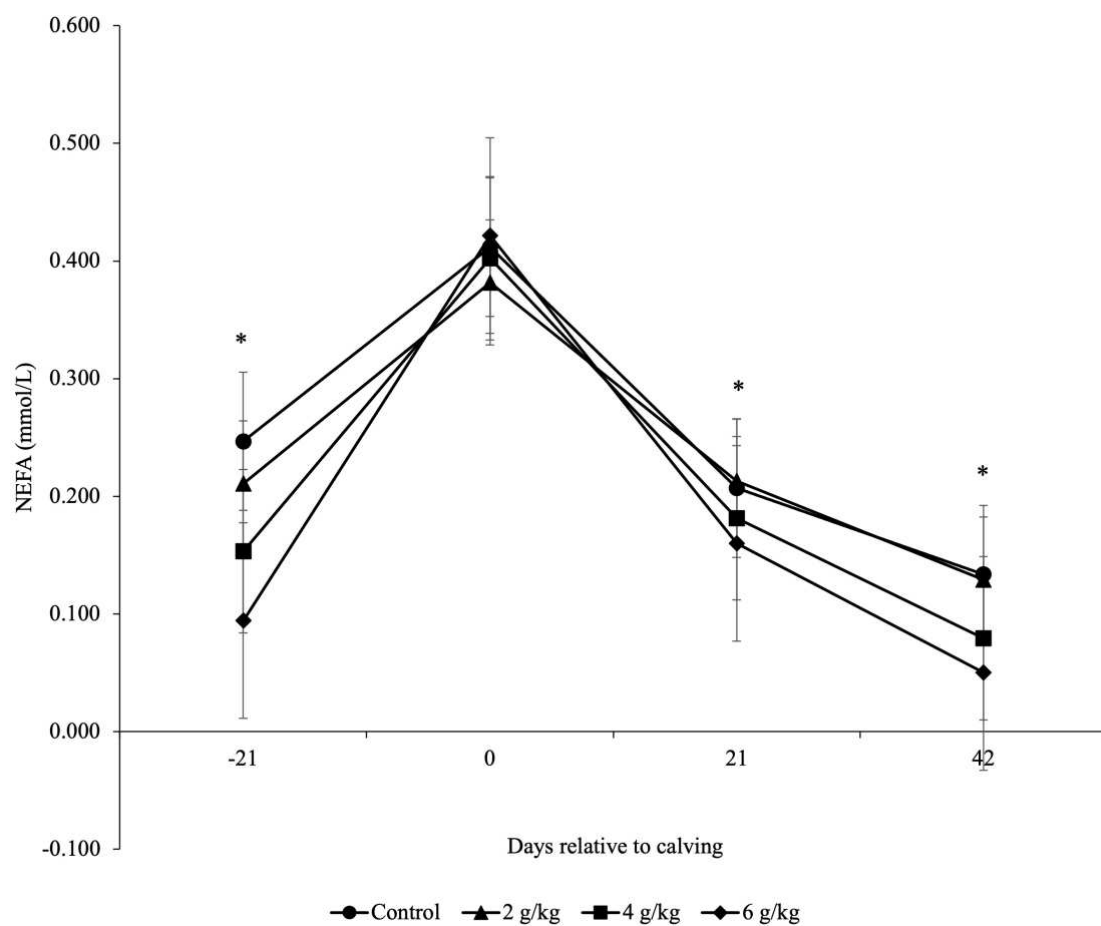


Figure 9- Concentrations of non-esterified fatty acids (NEFA) during pre and postpartum.

Asterisks (*) are significantly different between treatments ($P < 0.05$)