UNIVERSIDADE FEDERAL DE VIÇOSA

MARIANA DA NATIVIDADE FERREIRA VITAL

METABOLIC, REPRODUCTIVE, AND PERFORMANCE EVALUATION IN PRIMIPAROUS NELLORE BEEF COWS UNDER GRAZING WITH DIFFERENT AGES AT BREEDING

VIÇOSA – MINAS GERAIS
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Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Cláudia Batista Sampaio

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ABSTRACT

VITAL, Mariana da Natividade Ferreira, M.Sc., Universidade Federal de Viçosa, February, 2021. **Metabolic, reproductive, and performance evaluation in primiparous Nellore beef cows under grazing with different ages at breeding.** Adviser: Claudia Batista Sampaio.

The objective of this study was to evaluate the metabolic, reproductive and performance differences of primiparous Nellore cows, which became pregnant at 14 or 24 months. Thirty-eight primiparous Nellore cows with 200 ± 30 days of gestation were used, divided into two treatments: 14 - animals that became pregnant at 14 months of age, and 24 - animals that became pregnant at 24 months of age. The animals received 1.5 kg / day of supplement (36% CP) from prepartum until the end of the breeding season, after this period, cows and calves received mineral mixture ad libitum. To evaluate productive performance, cows were weighed and evaluated for body condition score (BSC), and calves were weighed at birth, at 45, 95,160, 220 days and at weaning. Blood samples were taken on the average 15 days before parturition, and on days 30, 60, 120, 240 days postpartum to measure glucose, total proteins, albumin, urea, beta-hydroxybutyrate (β-OHB) and non-esterified fatty acids (NEFA), progesterone at 30 and 45 postpartum and for insulin-like growth factor-1 at prepartum, 60 and 240 days postpartum., Milk collections were done at 60, 120 and 240 days postpartum. The data were analyzed using the GLIMMIX procedures in the SAS, adopting 0.05 as a critical level of probability for type I error. There was interaction ($P<0.01$) between the factors gestation age and parturition data-point for body weight, average daily gain and milk yield and *Longissimus* muscle. There was effect of the parturition data-point ($P<0.01$) for the BSC and thickness of the *Longissimus* muscle. Glucose, urea, total proteins, albumin and non-esterified fatty acids were affected ($P<0.01$) by the parturition data-point. There was effect of the parturition time-point ($P<0.01$) for β-OHB. No difference was found ($P>0.05$) in the IGF-1 options, however a difference was observed for the progesterone motors ($P<0.01$). Parturition time-point affected ($P<0.01$) calf weights. There was no difference ($P>0.05$) for gestation age and parturition time-point. It was concluded that primiparous cows that became pregnant at 14 or 24 months of age have different nutritional and metabolic responses. However, cows that have become pregnant at 14 months will wean another calf during its productive life.

Keywords: Animal production. Beef cattle. Primiparous beef cows.

RESUMO

VITAL, Mariana da Natividade Ferreira, M.Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Avaliação metabólica, reprodutiva e de desempenho em vacas primíparas Nelore de corte sob pastejo com diferentes idades ao parto.** Orientadora: Cláudia Batista Sampaio.

O objetivo deste estudo foi avaliar as diferenças metabólicas, reprodutivas e de desempenho de vacas Nelore primíparas, que emprenharam aos 14 ou 24 meses. Foram utilizadas 38 vacas Nelore primíparas com 200 ± 30 dias de gestação, divididas em dois tratamentos: 14 - animais que emprenharam aos 14 meses de idade e 24 - animais que emprenharam aos 24 meses. Os animais receberam 1,5 kg / dia de suplemento (36% PB) do pré-parto até o final da estação de monta, após esse período vacas e bezerros receberam mistura mineral ad libitum. Para avaliação do desempenho produtivo, as vacas foram pesadas e avaliadas quanto ao escore de condição corporal (ECC), e os bezerros foram pesados ao nascer, aos 45, 95,160, 220 dias e ao desmame. Amostras de sangue foram coletadas em média 15 dias antes do parto e nos dias 30, 60, 120, 240 dias pós-parto para medir glicose, proteínas totais, albumina, uréia, beta-hidroxibutirato (β-OHB) e ácidos graxos não esterificados (NEFA), progesterona aos 30 e 45 pós-parto e para o fator de crescimento semelhante à insulina-1 no pré-parto, 60 e 240 dias pós-parto. As coletas de leite foram feitas aos 60, 120 e 240 dias pós-parto. Os dados foram analisados por meio dos procedimentos GLIMMIX do SAS, adotando-se 0,05 como nível crítico de probabilidade para erro tipo I. Houve interação (P<0,01) entre os fatores idade gestacional e dados-ponto de parto para peso corporal, ganho médio diário e produção de leite e músculo Longissimus. Houve efeito do dado-ponto de parto (P<0,01) para o BSC e espessura do músculo Longissimus. Glicose, uréia, proteínas totais, albumina e ácidos graxos não esterificados foram afetados (P<0,01) pelos dados de parto. Houve efeito tempo (P<0,01) para β-OHB. Nenhuma diferença foi encontrada (P>0,05) nas concentrações de IGF-1, entretanto foi observada diferença para as concentrações de progesterona (P<0,01). Houve diferença (P<0,01) par os pesos dos bezerros. Concluiu-se que vacas primíparas que emprenharam aos 14 ou 24 meses apresentam diferentes respostas nutricionais e metabólicas. No entanto, vacas que engravidam aos 14 meses desmamam outro bezerro durante sua vida produtiva.

Palavras-chave: Bovinos de corte. Produção animal. Vacas primíparas.

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Introduction

The majority of Brazilian beef cattle herds consist of Nellore animals raised on pasture, however, due to seasonal variations in forage quality and production, these animals usually reach puberty between 2 and 3 years of age (Latawiec et al., 2014 ; Nepomuceno et al., 2017). According to the Brazilian Association of Zebu Breeders, in 2020 the mean age at first calving in Nellore animals was 39 ± 6 months (ABCZ, 2020).

However, as long as genetic selection and nutritional management strategies are improved, heifers can start their reproductive life at 14 months of age (Eler et al., 2002; Nepomuceno et al., 2017).

The reduction in the age at which heifers reach puberty implies a reduction in the reproductive age intervals between calving, and also allows the identification and removal of young unproductive females from the herd (Menegaz et al., 2008).

The age at first birth is linked to the concept of “permanence”, which indicates the probability of the female remaining in the herd until 76 months of age, giving birth at least 3 times. In other words, the sooner this animal gives birth, more chances it will have to reach this goal. In 2020, this rate was 33.9% in Nellore females in Brazil (ABCZ, 2020).

Decreasing age at first calving from 3 to 2 years can generate an increase of 0.5 to 0.8 calf per cow during its productive life, and my 16% increase in the profitability of the cow-calf system (Ferraz, 2008).

However, improving the reproductive aspects of primiparous cows represents one of the biggest challenges for better efficiency in beef herds, as they generally have lower reproductive rates than multiparous cows. One of the main causes of this pattern is that heifers are still growing. Consequently, growth requirements are added to the regular requirements for maintenance and lactation (Spitzer et al., 1995; Boges, 2006; Freetly et al., 2006). Thus, due to the higher proportional energy demand, primiparous cows tend to return to ovarian function, on average, 20 to 40 days later than multiparous cows (Wiltbank, 1970; Resende et al., 2014).

Heifers that start their reproductive life at 24 months have more time to grow and reach the weight needed to be ready for calving than heifers that become pregnant at 14 months. However, as long as they are able to maintain growth, heifers that become pregnant at 14 or 24 months can have similar reproductive rates (Sá Filho, 2012).

Thus, this study aimed to evaluate metabolic, reproductive, and performance in primiparous nellore beef cows under grazing, who became pregnant at 14 or 24 months.

Material and methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (CEUAP-UFV, protocol no. 02/2020).

Animals handling, experimental design, and supplements

The experiment was conducted at Research, Learning and Extension Beef Cattle Unit in Universidade Federal de Viçosa,Viçosa, Minas Gerais state (20° 45′ S and 42° 52′ W). The experimental area is located in mountainous region, with 670 m of altitude, and presents annual precipitation of 1251 mm. The experiment lasted 343 days.

Thirty-eight Nellore primiparous beef cows at 200±30 days of gestation were divided into two treatments: 14 mo - animals that became pregnant at 14 months of age (body weight [BW] = 402.94±6.64 kg); or 24 mo- animals that became pregnant at 24 months of age (BW = 505.05±7.53 kg). The animals were divided into 7 paddocks, composed of two treatments according by their body weight.

The experimental area of -*Brachiaria decumbens*- pastures was divided into 7 paddocks of 3.2-ha for grazing with continuous stocking, equipped with water dispensers and feeders. Every 28 days, the animals were rotated between the paddocks to control any paddock effects on the treatments. All animals received 1.5 kg of supplement in a daily basis in order to meet 40% of the crude protein maintenance requirements according to recommendations of Nutrient Requirements of Zebu and Crossbred Cattle (BR-CORTE 2016). The total amount of supplement was infrequently delivered to the animals 3 times a week as follow: monday (3 kg), wednesday (3 kg), and friday (4.5 kg) at 11:00 h, from the beginning of the experiment (last third of gestation) until the last day of the breeding season. From this moment until weaning, the cow-calf pairs only had *ad libitum* access to a complete mineral mixture. The composition of supplement, mineral mix, and pasture are shown in Table 1 and 2.

Experimental procedures and sampling

Forage samples were taken every 28 days to evaluate total forage availability. In each paddock, five forage samples were randomly selected using a metal square (0.5 × 0.5 m) and cut at, approximately, 1 cm above the soil. Additionally, every 28 days, a hand-plucked sampling was performed to evaluate the chemical composition of the forage consumed by the animals. All samples were oven-dried (55°C), grounded with 1- and 2-mm knife mills (Willye® TE-680) for future analyses.

The animals were weighed at the beginning of the experiment (90 days before calving), at calving, and at weaning. Calf BW was recorded at birth, at 45, 95,160, 220 and 240 (weaning) days. The BW wwas ever measured at 0800 h, always without fasting; except on the day of parturition, when the cow-calf pair was weighed as soon as the birth was identified. The body condition score (BCS) was recorded at prepartum, at calving and at weaning by three experienced technicians on a scale ranging from 1 to 9.

At 15 days before calving and at weaning, *Longissimus* muscle area (LMA), *Longissimus* muscle depth (LMD), between the 12th and 13th ribs, transversal to the *Longissimus lombarum* muscle; fat thickness over the *Longissimus lombarum* (RBF), between the 12th and the 13th ribs, and fat thickness over the *gluteus biceps* (RPF) were recorded with an ultrasound (Aloka SSD 500; 3.5 MHz linear probe; Aloka Co. Ltd., Wallingford, CT, USA). Images were analysed in the BioSoft Toolbox® II for Beef (Biotronics Inc., Ames, Iowa, USA).

Blood samples were collected from jugular venous puncture 15 days before calving, and at 30, 60, 120 and 240 days postpartum, always on Thursdays, at 08:00 h to quantity non-esterified fatty acids (NEFA), β-hydroxybutyrate (β-OHB), total protein, albumin, and urea. On the 30 and 45 days postpartum there was also a blood sample for progesterone analysis; and on 15 days before calving, 60 and 240 days postpartum, samples for insulin-like growth factor-1 (IGF-1). Blood samples was collected into vacutainers with gel for serum separation and clot activation (BD Vacuntainer SST II Plus, São Paulo, Brazil) for analyses of progesterone, IGF-1, NEFA, β-OHB, total protein, albumin, and urea. For glucose analysis, samples were collected in tubes with ethylenediamine tetraacetic acid (EDTA) and sodium fluoride (BD Vacutainer Fluoreto/EDTA, São Paulo, Brazil). After collected, tubes were centrifuged at 3,600 × g for 15 min. Following centrifugation, the plasma and serum were collected and subsequently frozen (-20°C) for further analysis.

At 60, 120 and 240 days after calving, in addition to blood samples, milk samples were taken. The calves were separated from the mothers at 1500 h on the previous day. At 1730 h the calves were again placed with their mothers to suck all the milk, being separated again at 1800 h. After that, the cows returned to the paddocks whereas the calves remained in the cattle shed with free access to water. At 0600 of the following day, milking was performed mechanically. Milk secretion was stimulated with 1 mL of oxytocin (10 UI/ mL, Lactocina ®, Brazil) in the mammary artery, initiating milking immediately after oxytocin administration. After each milking, milk was weighed and registered. The milk produced was corrected for production in a 24-hours basis according to Almeida et al. (2018).

All cows were submitted to a fixed-time artificial insemination (FTAI) protocol 45 days after calving. For this, all cows received an intravaginal monodose implant (Primer®, Tecnopec, Brazil) containing 1.9 g progesterone (CIDR-B®, Pfizer Animal Health, Brazil) plus 2 mg of estradiol benzoate (Estrogin®, Farmavet, Brazil). Nine days after the beginning of the FTAI protocol, the implant was removed and 1.5 mL of equine chorionic gonadotropin (Ecegon®, Biogeneses Bago, Brazil) plus 2.0 mL of prostaglandin F2 alpha (Estron®, Agener União, Brazil) was administered. Twenty-four hours after the implant removal, 1 mL of estradiol benzoate (RIC-BE®, Tecnopec, Brazil) was administrated. Pre-ovulatory follicle diameter was measured by ultrasonography (DP-2200Vet® with a 7.5-MHz linear-array transrectal transducer; Mindray) 48 h after the implant removal. Cows that presented pre-ovulatory follicle's diameter larger than 11 mm were artificial inseminated. Conception and pregnancy rates were measured 30 days after the artificial insemination. A second FTAI protocol was performed in cows that did not present a positive pregnancy diagnosis in the first FTAI, as described.

Analyses

Supplement and forage samples were analyzed for DM (INCTCA G-003/1), ash (INCT-CA M001/1), crude protein (INCT-CA N-001/1), ether extract (INCT-CA G-004/1), and neutral detergent fiber (NDF) corrected for ash and protein (INCT-CA F-002/1) according to (Detmann et al., 2012). The non-fiber carbohydrates were calculated by using the following equation: NFC = 100 – (% CP + %NDF + %EE + %ash), where NFC = non-fiber carbohydrates; CP = crude protein content in the diet; NDF = neutral detergent fiber content in the diet; EE = ether extract content in the diet; and ash = ash content in the diet. indigestible neutral detergent fiber (iNDF) was processed at 2 mm and quantified by in situ incubation procedures with non-woven textile bags (100 g/m²) for 288 hours (Valente et al., 2011). The potentially digestible DM (pdDM; Paulino et al., 2008) was calculated by the following equation:

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

where pdDM = potentially digestible DM; NDF = neutral detergent fiber, iNDF = indigestible neutral detergent fiber.

The blood NEFA concentration was quantified by a colorimetric method, whereas blood β-OHB was analyzed by a enzymatic-kinetic method based on the oxidation of D-3-hydroxybutyrate to acetoacetate (Ref. Numbers FA115 and RB1007 respectively, Randox, Ireland, UK). Glucose (K082, Bioclin Quibasa, Belo Horizonte, Brazil) and urea (K056, Bioclin Quibasa, Brazil) were quantified by the enzymatic-colorimetric method; and total protein (K031, Bioclin Quibasa, Brazil); albumin (K040, Bioclin Quibasa, Brazil) was analysed by the colorimetric method. An automated biochemical analyser (Mindray BS 200E, Shenzhen, China) determined all the analyses previously mentioned. Serum concentrations of IGF- 1 were quantified by chemioluminescent assay in comercial in laboratory. Progesterone concentrations were measure by radioimmunoassay kit (125/RIA, ICN Pharmaceuticals, Inc, EUA) and quantified by in PerkinElmer-wizard -1470 automatic - gamma counter (Laboratory of Endocrinology/Physiology of Domestic Animals, UNESP, Araçatuba campus, Sao Paulo, Brazil).

Statistical analysis

The variables (with the exception of gestation days, IGF-1, average daily gain of calves [ADG], and conception rates at fixed time Ais) were analyzed as a completely randomized design, and the period was included as a repeated measure in the model:

$$Y_{ijkl} = \mu + T_i + \delta_{ij} + P_k + (T \times P)_{ij} + \varepsilon_{ijkl}$$

Where μ = general mean; T_i = fixed effect of the treatment i; δ_{ij} = random error with a mean of zero and variance of σ^2, the variance among animals within paddock, equal to the covariance among repeated measures within animals; P_k = fixed effect of period; $(T \times P)_{ik}$ =

fixed effect of the interaction between treatment i and period e; and ε_{ijkl} = random error with a mean of zero and variance of σ^2, the variance among measures between animals.

Seven variance–covariance structures (AR1, CS, UN, TOEP, VC, ARH1, TOEPH) were tested, and the ARH1 was the one that provided the best fit based on the Akaike information criterion.

Gestation days, IGF-1, ADG of calves, and conception rates at fixed time AIs were analyzed as a completely randomized design, model:

$$Y_{ij} = \mu + T_j + \varepsilon_{ij}$$

where μ = general mean; T_i = fixed effect of the treatment i; ε_{ij} = random error.

Data of conception rates at fixed time AIs were analyzed using binomial distribution.

All variables were analyzed using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4) and differences were declared significant at $P < 0.05$, and trends were declared when $P < 0.10$ as the critical level of probability for type I error.

Results

Milk yield was, on average, higher for 24-mo cows (P<0.01, Table 3), but decreased as days in milking increased (P<0.01) for both cow ages (Figure 1). The BW of the cows presented an interaction between age and time (P<0.01). On average, 24-mo cows were heavier (P<0.01) The slicing of interaction indicated that both categories presented an intense BW loss at parturition demonstrated by the drop in the BCS as well (Figure 1). However, from that BW of 24-mo cows remained stable (P>0.05), whereas 14-mo cows presented a gradual BW recovery after parturition (P<0.05), due to the higher GMD of these animals (Table 3).

The weight of calves born to 24-mo cows was lower at birth, however it was higher at weaning (P<0.01) and this difference was significant from 160 days of age (Figure 1.C).

There were no effects on 12th rib fat and rump fat; however, LMD and LMA values were higher ($P<0.001$) for 24-mo cows (Table 3, Figure 3).

Among blood metabolites, glucose, urea, total protein, albumin and NEFA were affected ($P<0.01$) by the evaluation period in animals of both ages, however, no significant difference was observed ($P>0.05$) for IGF-1 concentrations (Table 4). In addition, 24-mo cows had the highest concentration ($P<0.01$) of β-OHB at prepartum and 30 days after calving (Figure 4). The 24-mo cows also had a higher concentration of progesterone ($P<0.01$, Table 4).

There was no difference ($P>0.05$) for pregnancy rates and gestation days between 14 and 24-mo cows (Table 5).

Discussion

Primiparous that became pregnant at 24 months in the beginning of the experiment were upper weight, which is expected since these animals were in a more advanced stage of development (Bitencourt et al., 2020). The cows that became pregnant at 14 months reached about 80% of the adult weight at parturition, this being the recommended weight target for the animal to be able to maintain the nutritional requirements at postpartum and, consequently, return to reproduction (Rovira, 1981). The continued weight loss after parturition by the 24-mo cows, associated with the greater milk yield of these animals (21.5% greater than that of treatment 14) demonstrate the greater energy demand of these animals for lactation.

Although the results of this study show values for milk production after 60 days postpartum, a greater milk production before this period can be demonstrated due to the higher average daily gain presented by calves born from the 24-mo cows. These calves had birth weight 16% lower than the calves of 14-mo cows, and at 45 days of age they were already heavier. The average daily gain of calves born to 24-mo cows was greater throughout the period, obviously due to higher milk production. The significant difference found after 160 days in weight (Figure 1.C) may be related to the greater persistence of lactation in animals from treatment 24, which had a smaller reduction in milk production between days 120 and 240 postpartum.

In beef cows, the BCS is a determining factor for the return of cows to estrus, improving the conception rates. According to Bohnert (2013), cows that maintained their BCS between 5 and 6 in the last trimester of pregnancy lost less BW and had a lower decrease in BCS at parturition, in addition to having higher pregnancy rates in the next breeding season when compared to cows that had BCS between 4 and 5.

All animals kept with BCS suitable for reproduction during the whole experiment, even in the parturition when they had a decrease of 1 point in the score, that is, between 5 and 6 (Spitzer et al., 1995). Even 24-mo animals having a lower average daily gain, the fact that they have adequate BCS may have guaranteed sufficient body reserves to not compromise their reproduction (Diskin and Kenny, 2016). These results demonstrate the importance of ensuring proper nutrition during pregnancy, so that the animals maintain an adequate BCS, improving their reproductive performance (Marques et al., 2016).

Ultrasonography, like BCS, is an indicator of how the animal's body reserves are, and this is more sensitive to small changes in body composition than BCS (Schröder and Staufenbiel, 2006). The reduction in LMA and LMD values at weaning in relation to prepartum suggest that these animals had a protein mobilization (Figure 3). These results are justified by the greater fetal demand for amino acids at the end of gestation and the beginning of lactation (BR-CORTE, 2016). When the protein demand is greater or close to the amount ingested, there is mobilization of muscle tissue to maintain the necessary levels of amino acids circulating (Bell et al., 2000).

NEFA concentrations were higher in 15 days before calving, indicating an increase in the lipolysis rate of adipose tissue (Lopes et al., 2016). However, they decreased throughout the experiment, suggesting that the animals recovered their nutritional status.

Indicators of the mobilization of lipid reserves β-OHB and NEFA have being related to the energy deficit (González, 2000). High plasma levels of β-OHB are linked to energy deficit at the end of pregnancy and the beginning of lactation (Russel and Wright, 1983). The high levels of β-OHB, together with the higher milk yield of the 24-mo cows can be justified by the peak lactation of Nellore cows, which occurs in the first postpartum month (Espasandin et al., 2016) suggesting a greater mobilization of tissue to supply the needs of lactation.

The results of ultrasonography, in addition with the results of β-OHB and the non-recovery of body weight after calving, demonstrate that the 24-mo cows had a higher protein and energy demand; this greater demand can be justified by its greater milk production in relation to the 14-mo cows (Freetly et al., 2006).

The progesterone concentration was higher for 24-mo cows (Table 4) and the pregnancy rate in 14-mo cows increased by 50% after the second FTAI protocol, while in 24-mo cows this difference was 30%. These results show that a greater number of younger animals took longer to cycle again, showing lower sexual maturity of these animals.

In general, calves born from primiparous cows are lighter than the calves of multiparous cows (Day and Nogueira, 2013). In this study, calves born to 14-mo cows had a weaning weight of around 20 kg lower than those born to 24mo cows due to less milk production by their mothers. A management option to make up for this difference would be the use of creep-feeding to these animals which would increase by around 0.100g/day the average daily gain, making these animals to be weaned at the same weight as the calves of the treatment 24 cows (Carvalho et al., 2019).

In summary, our data support the conclusion that primiparous cows that become pregnant at 14 or 24 months of age have different nutritional and metabolic responses. The 14-mo cows weaned lighter calves, however, this animals will have delivered an extra calf in their productive life when compared to the 24-mo cows.

Table 1. Chemical composition of the supplement	
Ingredients (%; as-fed basis)	
Corn meal	70
Soybean meal	21
Urea	5.4
Ammonium sulfate	0.6
Mineral mixture[a]	3
Chemical composition (g/kg of DM)	
Organic matter	945.3
Crude protein	362.8
Ether extract	10.7
apNDF[b]	164.8

apNDF, Neutral detergent fiber corrected for ash and protein residue.
[a]Mineral mixture: 50% dicalcium phosphate, 47.2% sodium chloride, 1.5% zinc sulfate, 0.7% copper sulfate, 0.05% cobalt sulfate, 0.05% potassium iodate, and 0.5% manganese sulfate.

Table 2. Chemical composition of the forage

Item	Period of sampling				
	Prepartum	+30	+60	+120	+240
DM (g/kg)	511.5	289.8	278.4	277.3	298.3
OM (g/kg)	936.9	895.7	906.4	894.0	894.5
CP (g/kg)	51.3	131.1	140.0	124.7	118.4
EE (g/kg)	16.9	21.9	29.4	33.7	29.7
apNDF (g/kg)	684.3	609.2	568.3	570.4	608.7
NFC (g/kg)	170.5	146.1	150.6	144.4	165.9
iNDF (g/kg)	268.9	273.8	137.5	134.7	179.3
pdDM (kg/ha)	4945.42	9980.23	7916.58	8100.38	6282.80

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

Table 3. Cows and calves performance and milk production

Item	Treatment[a] 14	Treatment[a] 24	SEM[b]	P-value Treatment	P-value Time	P-value Treatment × Time
Cows						
MY (kg/d)						
60 days postpartum	6.46	8.16	0.35			
120 days postpartum	4.52	5.52	0.20	0.001	0.001	0.001
240 days postpartum	3.66	4.96	0.22			
BCS (scale 1-9)						
Prepartum	6.4	6.65	0.09			
Parturition	5.40	5.52	0.10	0.197	0.001	0.691
Weaning	5.96	6.01	0.15			
BW (kg)						
Prepartum	402.94	505.05	6.53			
Postpartum	387.08	447.06	5.85	0.001	0.001	0.001
Weaning	397.22	452.57	5.92			
ADG (kg/d)						
Prepartum	0.365	0.296	0.04	0.001	0.001	0.001
Postpartum	0.129	0.015	0.02	0.001	0.001	0.001
LMA (cm^2)						
Prepartum	41.12	48.74	3.83	0.028	0.001	0.001
Postpartum	39.76	38.56	3.93			
LMD (mm)						
Prepartum	69.58	79.10	1.99	0.001	0.001	0.066
Postpartum	66.30	68.41	1.78			
RBF (mm)						
Prepartum	2.14	2.50	0.22	0.126	0.012	0.734
Postpartum	1.78	2.03	0.26			
Rump fat (mm)						
Prepartum	2.79	4.02	0.24	0.006	0.012	0.317
Postpartum	2.30	2.92	0.34			
Calves						
BW (kg)						
Birth	27.12	22.25	13.13	0.103	0.001	0.006
Weaning	181.73	203.53	12.76			
ADG (kg/d)	0.642	0.727	0.023	0.001	-	-

My, milk yield; BCS, body condition score; BW, Body Weight; ADG, average Daily gain; LMA, *Longissimus* muscle area, LMD, *Longissimus* muscle Delph; RBF, 12-th rib fat.

[a] Treatment: 14 - animals that became pregnant at 14 months of age; 24 - animals that became pregnant at 24 months of age.

[b] Standard error of mean.

Table 4. Blood parameters

Item	Period of sampling X Treatment[a]												SEM[b]	*P-value*		
	Prepartum		+30		+45		+60		+120		+240			Treatment	Time	Treatment × Time
	14	24	14	24	14	24	14	24	14	24	14	24				
Glucose (mg/dL)	61.56	61.47	65.76	68.08	-	-	65.38	68.22	67.02	68.56	68.11	71.00	3.24	0.297	0.001	0.753
Urea (mg/dL)	27.12	32.94	33.96	37.42	-	-	24.87	28.48	18.93	21.76	20.99	21.20	0.910	0.004	0.001	0.213
Total protein (g/dL)	7.38	7.56	8.08	8.36	-	-	7.88	8.03	8.47	8.57	9.01	8.82	1.23	0.472	0.001	0.365
Albumin (g/dL)	3.28	3.35	3.21	3.19	-	-	3.07	3.00	3.14	3.08	3.05	3.04	0.031	0.729	0.001	0.302
NEFA (mmol/L)	0.429	0.529	0.206	0.316	-	-	0.127	0.135	0.224	0.196	0.108	0.157	0.022	0.161	0.001	0.330
β-OHB (mmol/L)	0.542	0.718	0.411	0.514	-	-	0.438	0.421	0.508	0.482	0.560	0.578	0.019	0.075	0.001	0.045
Progesterone (ng/mL)	-	-	0.026	0.741	0.035	0.830	-	-	-	-	-	-	0.191	0.009	0.857	0.882
IGF-1 (ng/mL)	125.1	125.9	-	-	-	-	129.0	149.3	-	-	120.9	139.8	6.94	0.203	0.112	0.352

NEFA, Non-esterified fatty acids; β-OHB, β-hydroxybutyrate IGF-1, insulin-like growth factor-1.
[a] Treatment: 14 - animals that became pregnant at 14 months of age; 24 - animals that became pregnant at 24 months of age .
[b] Standard error of mean.

Table 5. Pregnancy rates and gestation days

	Treatment[a]			
	14	24	SEM	*P-value*
Pregnancy rate, FTAI 1 (%)	35.00	52.94	0.11	0.283
Pregnancy rate, FTAI 2 (%)	70.00	82.35	0.09	0.393
Gestation Days	291.17	294.36	1.20	0.091

[a]Treatment: 14 - animals that became pregnant at 14 months of age; 24 - animals that became pregnant at 24 months of age.

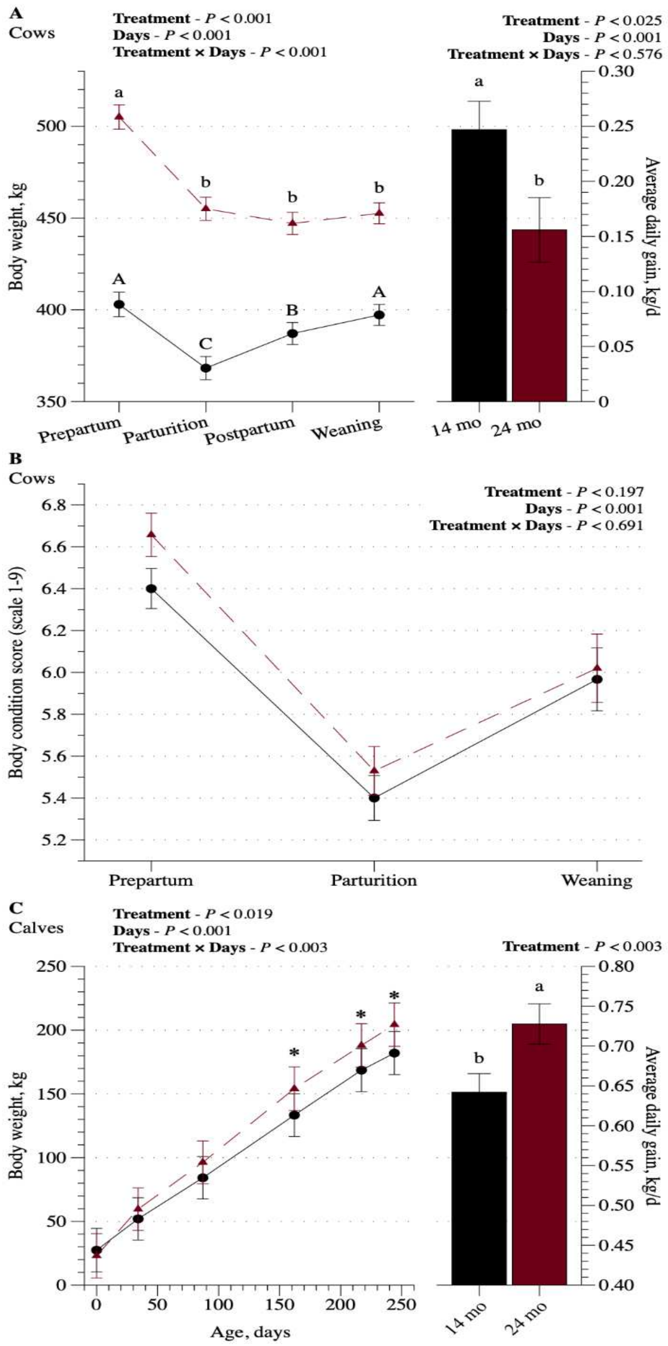

Figure 1. Cows body weight and average daily gain (A), cows body score condition (B), calves body weight and average daily gain (C).

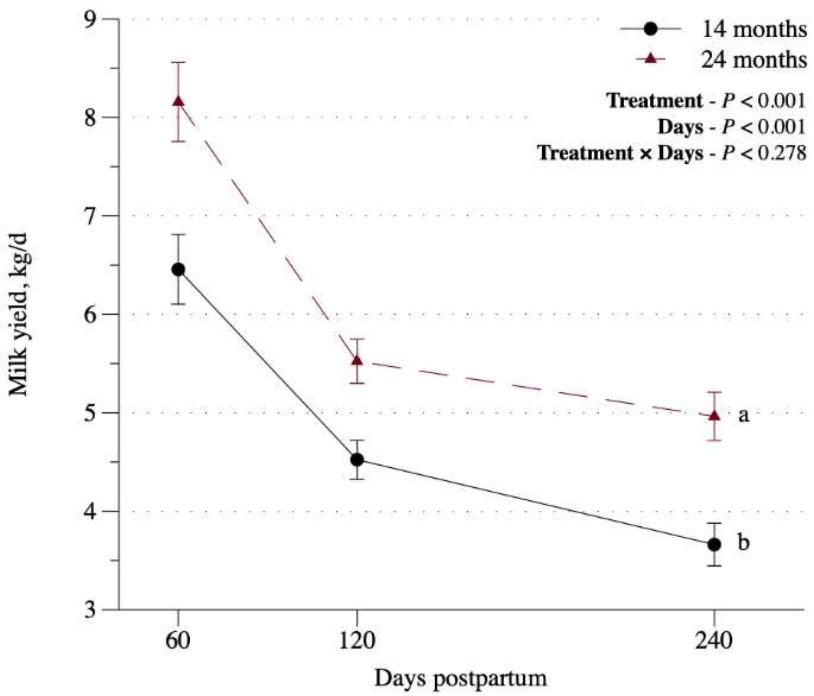

Figure 2. Milk yield at 60, 120 and 240 days postpartum.

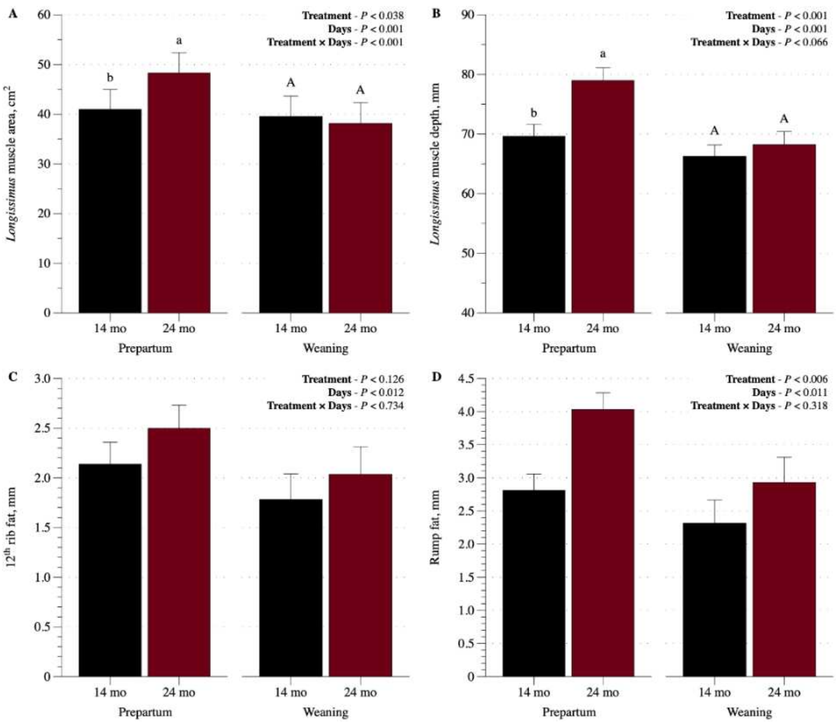

Figure 3. *Longissimus* muscle area and delph, 12-th rib and rump fat at prepartum and at weaning.

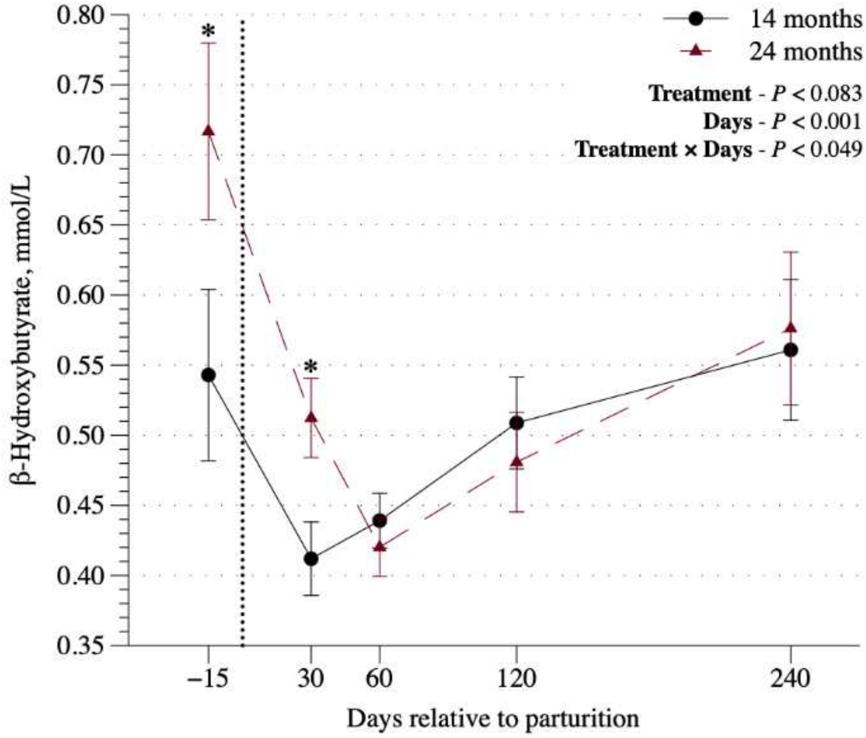

Figure 4. β-hydroxybutyrate concentration at 15 days before parturition, 30, 60,120 and 240 days postpartum.

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