FELIPE HENRIQUE DE MOURA

EFFECTS OF ENERGY-PROTEIN SUPPLEMENTATION FREQUENCY ON PERFORMANCE OF PRIMIPAROUS GRAZING BEEF COWS DURING PRE AND POSTPARTUM

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.*

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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*.

APPROVED: February 21, 2018.

Mário Fonseca Paulino

Luciana Navajas Rennó

Edenio Detmann
(Co-adviser)

Mozart Alves Fonseca

Marcio de Souza Duarte
(Adviser)

"To live is to adapt."

Euclides da Cunha

Os Sertões

To my parents, for their love, work and encouraging examples.
To my brother, for his love and friendship.
To my grandfather, for his passion for cattle.
To my relatives, for always being by my side, giving me strength and praying!

I dedicate.

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Physiology/Reproduction and Animal's Food Factory. Thank you for your help in conducting the fieldwork, collaboration in laboratory analysis and fellowship.

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BIOGRAPHY

FELIPE HENRIQUE DE MOURA, son of Carlos Alberto Moura and Lenilde Cândida de Moura, was born in Divinópolis/MG-Brazil on December 4, 1992.

De Moura started the undergrad in Animal Science at Universidade Federal de Viçosa in 2011 and became a Bachelor of Science in Animal Science in 2016. At the same year he started the M.Sc. program with major on ruminant nutrition and beef cattle production, with sandwich period at the University of Nevada-Reno in Reno/NV, USA.

On February 21th of 2018, De Moura defended his master's dissertation to obtain the *Magister Scientiae* degree in Animal Science.

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ABSTRACT

MOURA, Felipe Henrique de, M.Sc., Universidade Federal de Viçosa, February, 2018. **Effects of energy-protein supplementation frequency on performance of primiparous grazing beef cows during pre and postpartum.** Adviser: Marcio de Souza Duarte. Co-adviser: Edenio Detmann.

The present study was performed to evaluate the effects of energy-protein supplementation and supplementation frequency during pre (105 d before calving) and postpartum (105 d after calving) of primiparous beef cows on performance and metabolic characteristics under grazing conditions. Twenty-four pregnant Nellore primiparous cows with average BW of 409±8.0 kg, 22±0.9 mo old and 172±2.5 d of gestation were used in this trial. Treatments were randomly assigned to primiparous cows: Control (*ad libitum* mineral mix), Daily Supplementation (1.5 kg/d of concentrate/animal) and Infrequent Supplementation (4.5 kg of concentrate/animal every three days). The BW, ADG, BCS, fat-thickness and ribeye area (RA) were evaluated at pre and postpartum period. Two 9-d intake-digestibility trials were performed throughout the experimental period where the first at 55 d before parturition, and the second at 55 d after parturition. Concentrations of glucose, IGF-1, NEFA, β-OH, cholesterol and triglycerides were used as indicators of energy *status*, and free amino acids (AA), serum urea nitrogen (SUN), total protein, albumin and globulins were used as indicators of proteic *status* collected at 27 and 9 d before parturition, at the day of parturition, 9 and 27 d after parturition. Globulins were calculated subtracting the albumin quantified from the total protein level. Biopsies of hepatic and skeletal muscle tissues were performed at 27 d prior to calving to evaluate ureagenesis and energy metabolism biomarkers. Serum progesterone (P4) level was evaluated at the 36^{th} d postpartum. The response variables were analyzed using PROC MIXED of SAS 9.4 (SAS Inst., Cary, NC). The frequency of supplementation did not alter ($P > 0.10$) BW, adjusted BW at day of parturition (adjBW), BW after calving upon parturition (calvingBW), ADG, BCS, RA and fat-thickness during pre and postpartum. The BW ($P = 0.079$), adjBW ($P = 0.078$) and ADG ($P = 0.074$) were higher for supplemented cows during the prepartum. The BCS ($P = 0.251$), RA ($P = 0.352$), fat-thickness on longissimus muscle ($P = 0.199$) and on *Biceps femoris* muscle ($P = 0.924$) were not affect by supplementation during the prepartum. At 105 d after calving the supplementation did not affect ($P > 0.10$) BW, ADG, BCS, RA, and fat-thickness. Birth

BW of calves, ADG and calf BW 105 d after calving were not different ($P > 0.10$) according to supplementation and frequency of supplementation. The total dry matter intake and forage voluntary intake were not affected by supplementation and supplementation frequency during prepartum ($P > 0.10$) and postpartum ($P > 0.10$). Daily supplemented animals had higher ($P < 0.001$) glucose levels than animals supplemented every three days. Interaction between treatment and sampling time was significant for AA ($P < 0.001$), glucogenic AA ($P < 0.001$), ketogenic AA ($P < 0.001$), gluco/ketogenic AA ($P < 0.001$), and SUN ($P = 0.005$). The supplementation and frequency of supplementation did not alter ($P > 0.10$) the levels of IGF-1, NEFA, β-OH, cholesterol and triglycerides. Likewise, the supplementation and frequency of supplementation did not alter ($P > 0.10$) the levels of total protein, albumin and globulins. The protein abundance of carbamoyl phosphate synthase-1 (CPS-1), mRNA levels of carnitine palmitoyltransferase-1 (CPT-1) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) were not affected ($P > 0.10$) by supplementation and supplementation frequency. Similar percentage ($P = 0.606$) of cows with ovarian activity (P4 > 1 ng/ml) and conception rate ($P = 0.842$) were observed among the treatments. The energy-protein supplementation during pre and postpartum of primiparous beef cows under grazing conditions do not improve performance and metabolic characteristics. However, the reduction of frequency of supplementation do not result negative effects on performance and metabolic characteristics.

RESUMO

MOURA, Felipe Henrique de, M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Efeitos da frequência de suplementação proteica-enegética sobre o desempenho de vacas primíparas de corte em pastejo durante o pré e pós-parto.** Orientador: Marcio de Souza Duarte. Coorientador: Edenio Detmann.

O presente estudo foi realizado para avaliar os efeitos da suplementação proteico-enegética e frequência de suplementação durante o pré (105 dias antes do parto) e pós-parto (105 dias após o parto) sobre o desempenho e características metabólicas de vacas primíparas de corte em pastejo. Foram utilizadas vinte e quatro vacas primíparas Nelore com PC médio de 409±8.0 kg, 22±0.9 meses de idade e 172±2.5 dias de gestação. Os tratamentos foram distribuídos aleatoriamente aos animais: Controle (mistura mineral *ad libitum*), Suplementação Diária (1.5 kg/dia de concentrado/animal) e Suplementação Infrequente (4.5 kg de concentrado/animal a cada três dias). O PC, GMD, ECC, esspessura de gordura subcutânea (EGS) e área de olho de lombo (AOL) foram avaliados no pré e pós-parto. Foram realizados dois ensaios de consumo e digestibilidade, o primeiro aos 55 dias antes do parto e o segundo, 55 dias após o parto. Amostras de sangue foram coletadas nos dias 27 e 9 dias antes do parto, ao parto, 9 e 27 dias após o parto para mensuração das concentrações de glicose, IGF-1, AGNE, β-OH, colesterol e triglicerídeos, utilizados como indicadores do *status* energético, e mensuração de aminoácidos livres (AA), nitrogênio ureico sérico (NUS), proteína total, albumina e globulinas utilizados como indicadores de *status* proteico dos animais. As globulinas foram calculadas subtraindo a albumina quantificada do nível de proteína total. Aos 27 dias antes do parto, foi realizado biópsias de tecido hepático e tecido muscular para avaliar biomarcadores de ureogênese e metabolismo energético. Concentração sérica de progesterona (P4) foi avaliada no 36º dia pós-parto. Todas variáveis foram analisadas utilizando PROC MIXED do SAS 9.4 (SAS Inst., Cary, NC). A frequência de suplementação não alterou ($P > 0.10$) o PC, PC ajustado ao dia do parto (PCajus), PC após o parto (PCp), GMD, ECC, AOL e EGS no pré e pós parto. O PC ($P = 0.079$), PCajus ($P = 0.078$), e GMD ($P = 0.074$) foram maior para as vacas suplementadas no pré-parto. O ECC ($P = 0.251$), a AOL ($P = 0.352$), a EGS sobre o músculo longissimus ($P = 0.199$) e sobre o músculo *Biceps femoris* ($P = 0.924$) não foram afetados pela suplementação durante o pré-parto. Aos 105 dias após o parto, a suplementação não afetou ($P > 0.10$) o PC, GMD, ECC, AOL e EGS. O PC dos

bezerros ao nascer, GMD e PC dos bezerros 105 dias após o parto não foram diferente ($P > 0.10$) de acordo com a suplementação e a frequência de suplementação. O consumo de matéria seca total e o consumo voluntário de forragem não foram afetados pela suplementação e frequência de suplementação durante o pré-parto ($P > 0.10$) e pós-parto ($P > 0.10$). Os animais suplementados diariamente apresentaram maiores níveis de glicose ($P < 0.001$) que os animais suplementados a cada três dias. Interação entre tratamento e tempo de coleta foi significativa para AA ($P < 0.001$), AA glicogênicos ($P < 0.001$), AA cetogênicos ($P < 0.001$), AA glico-cetogênicos ($P < 0.001$) e NUS ($P = 0.005$). A suplementação e frequência de suplementação não alteraram ($P > 0.10$) os níveis séricos de IGF-1, AGNE, β-OH, colesterol e triglicerídeos. Do mesmo modo, a suplementação e frequência de suplementação não alteraram ($P > 0.10$) os níveis de proteína total, albumina e globulinas. A abundância da proteína carbamoil-fosfato sintetase-1 (CPS-1), os níveis de mRNA de carnitina palmitoyltransferase-1 (CPT-1) e do coativador-1α do receptor γ ativado por proliferador de peroxissoma (PGC-1α) não foram afetados ($P > 0.10$) pela suplementação e frequência de suplementação. Observou-se uma porcentagem semelhante ($P = 0.606$) de vacas com atividade ovariana (P4 > 1 ng/ml) e taxa de concepção ($P = 0.842$) entre os tratamentos. A suplementação proteica-energética durante o pré e pós-parto de vacas primíparas de corte em pastejo não melhora o desempenho e características metabólicas. Contudo, a redução da frequência de suplementação não resulta em efeitos negativos sobre o desempenho e características metabólicas.

INTRODUCTION

The most important factors that limits the production efficiency of a cow-calf enterprise are reproduction and nutrition (Hess et al., 2005; Silva et al., 2017). Thus, due to additional demands for growth combined with stresses of first gestation and lactation that are often not fulfilled at grazing systems, primiparous cows have contributed for production inefficiency (Spitzer et al., 1995; Ciccioli et al., 2003; Mulliniks et al., 2012). Therefore, energy-protein supplementation for primiparous cows is required to reproductive success (Funston, 2008).

However, supplementation programs substantially increase production cost in beef cattle systems, including expenses associated with feed purchase and labor required for daily supplement feeding (Miller et al., 2001). Therefore, feeding strategies that reduce labor for supplement feeding, such as provide supplement every three days, can be attractive to cow-calf producers to reduce feeding costs, but mainly due positive impact on reproductive performance of beef primiparous cows. The nitrogen recycling capacity of ruminants has been reported as the main biological mechanism that allows the use of infrequent supplementation (Krehbiel et al., 1998; Bohnert et al., 2002; Atkinson et al., 2010). This biological event can be significant even in *medium-high* quality forage (Batista et al., 2017a; Batista et al., 2017b).

Thus, we hypothesized that the energy-protein supplementation improves the animal performance and metabolic characteristics, and that frequency of supplementation can be reduced without impacting performance and metabolic characteristics of beef primiparous cows. The aim of the current study was to evaluate the effects of energy-protein supplementation and supplementation frequency during pre (105 d from parturition) and postpartum (105 d after parturition) on performance and metabolic characteristics of grazing primiparous beef cows.

MATERIAL AND METHODS

All animal care procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 19/2017)

Experimental area

The experiment was carried out at the Department of Animal Science of the Universidade Federal de Viçosa, MG, Brazil, (20° 45′ S, 42° 52′ W), from June to December 2015, over the dry season, dry-to-rainy transition season, and rainy season. The experimental area was located in a hilly region, at altitude of 670 m. The climate of the area was classified according to Köppen-Geiger (Kottek et al., 2006) as Cwa (humid temperate, with dry winter, hot summer), with the average temperature of the coldest month (June) lower than 18ºC and that of the hottest month (February) higher than 22ºC. Data collection regarding the average among minimum and maximum temperatures and precipitation were collected throughout the experimental period (Figure 1).

The experimental area had a total of 24-ha divided in six paddocks for grazing with continuous stocking. Each group was managed on a 4-ha paddock with *Brachiaria decumbens* pastures with available water and feed throughs. The pastures were reserved during sixty days prior to the start of the trial, in order to provide a non-restrictive forage amount for feed intake. Due to infrequent supplementation occurs every three days, all activities were performed on multiple days of three.

Animals, treatments and experimental design

Twenty-four pregnant Nellore primiparous cows with average BW of 409±8.0 kg, 22±0.9 mo old and 172±2.5 d of gestation were used during pre and postpartum period.

Animals were adapted to basal supplementation and experimental area for 14 days. After the adaptation period, the animals were kept in the same paddocks for 210 days, being 105 days prior to parturition and 105 days after parturition.

The amount of supplement (1.5 kg/cow) and quantity of crude protein (450 g/d) were assigned to meet protein requirements of primiparous cows, with an average gain daily of 0.6 kg/d at pasture containing 60 g/kg of crude protein (dry matter basis) in order to achieve mature weight of female zebu cattle according to BR-CORTE (2016). Feeds and chemical composition of experimental supplement are shown in the Table 1.

Treatments were randomly assigned to primiparous cows. The experimental treatments were: control (*ad libitum* mineral mix); daily supplementation (1.5 kg/d of supplement and *ad libitum* mineral mix), fed once a day at 1100h; infrequent supplementation (4.5 kg of supplement every three days and *ad libitum* mineral mix), fed once a day at the day of supplementation 1100h.

Animal data collection

The animals were weighed at the beginning of the trial (105 d before calving), 15 d before parturition, at the day of parturition upon calving, and at the end of the trial (105 d after calving). Body weights were obtained at 0600h, except on the day of parturition. Calf BW was also recorded at birth and at 105 d after parturition.

At the beginning of the trial, 15 d before parturition and at end of the experiment, ultrasound images were taken using a Aloka SSD 500 ultrasound provided with a 3.5 MHz linear probe (Aloka Co. Ltd., Wallingford, CT). Images were taken at longissimus muscle located between the 12^{th} to 13^{th}-ribs for measurements of backfat thickness and ribeye area, and at *Biceps femoris* muscle for measurement of fat-thickness. After that, the images were analyzed in the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, Iowa, USA). Body condition scores (BCS) were also

recorded on a scale ranging from 1 to 9 according to NRC (1996) by 3 experienced technicians; all evaluations were performed by the same three evaluators.

Feed sampling and chemical analysis

Representative samples of supplement were collected monthly. Pasture samples composition were obtained by hand-clipping every 2 weeks. Once a month, a second pasture sample was collected from each paddock. The second samples were constituted of four forage subsamples randomly selected using a metal (0.5 x 0.5m) square and clipped approximately 1 cm above the ground to estimate the potentially digestible forage dry matter (pdDM) availability according to Paulino et al. (2008a). Samples of supplement and pasture were oven-dried (55°C) and grounded in a Wiley mill (model 3; Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen.

The pdDM was estimated using the second pasture sample collected in each period as previously described, using the following equation:

$$pdDM(\%; dry\ matter\ basis) = 0.98x(100 - NDF) + (NDF - iNDF)$$

Where, 0.98 represents the true digestibility coefficient of forage cell content; NDF represents the neutral detergent fiber assayed with a heat stable amylase; and iNDF is the forage content of indigestible neutral detergent fiber.

Two 9-d intake-digestibility trials were performed throughout the experimental period where the first at 55 d before parturition, and the second at 55 d after parturition. Titanium dioxide (TiO_2) was used as external marker to estimate fecal excretion. Twenty grams of TiO_2 by animal was packaged in paper cartridges and delivered via the esophagus with a metal probe, once daily at 1030h over nine days. Six days were allowed for stabilization of external marker excretion, and fecal samples were then subsequently collected at 0800h and 1500h on the seventh day, at 1000h and 1700h on the eighth day, and at 0600h and 1300h on the ninth day of the intake trial.

Approximately 300 g of fecal samples were collected immediately after spontaneous defecation. Each fecal sample was oven-dried (55°C) and grounded as described for pasture. Grounded samples were proportionally mixed to make a composted sample per animal on the pre and postpartum.

Pooled samples of each material ground through 1-mm sieves (supplement, pasture, and feces) were analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012) for DM (dried overnight at 105°C; method INCT-CA number G-003/1), ash (complete combustion in a muffle furnace at 600°C for 4 h; method INCT-CA number M-001/1), N (Kjeldahl procedure; method INCT-CA number N-001/1), NDF corrected for ash and protein (using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein; method INCT-CA number F-002/1). The fecal samples were also analyzed for levels of TiO2 by colorimetry (method INCT-CA M-007/1). From samples of supplement, pasture, and feces processed through a 2-mm sieve, iNDF content was determined as the residual NDF remaining after 288 h of ruminal in situ incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY), according to Valente et al. (2011).

Fecal excretion (FE) was estimated by the ratio of TiO_2 and its concentration in the feces, as per the following equation:

$$FE\ \left(\frac{g}{d}\right) = TiO_2\ \div [TiO_{2feces}]$$

Where, FE is the fecal excretion (g/d); TiO_2 is the amount of TiO_2 (g/dia); TiO_{2feces} is the fecal concentration of the external indicator in the feces (g/g).

Dry matter (DM) intake and the forage voluntary intake (FDM) were estimated by using the iNDF as an internal marker and calculated by the following equations:

$$DM\ \left(\frac{kg}{d}\right) = \left[\left(\left(FE\ x\ iNDF_{feces}\right) -\ iNDF_{sup}\right) \div\ iNDF_{forage}\ \right] + DMSI$$

Where, FE is the fecal excretion (kg/d); $iNDF_{feces}$ is the concentration of iNDF in the feces (kg/kg); $iNDF_{sup}$ is the iNDF in the supplement (kg); $iNDF_{forage}$ is the concentration of iNDF in forage (kg/kg) and DMSI is DM supplement intake (kg).

Blood hormone and metabolite assessment

Blood samples were collected by puncture of the jugular vein only at one single day, after 3 days of the infrequent supplementation and before the next infrequent supplementation at 0700h on 27 d and 9 d prior to parturition, at calving day, 9 d and 27 d after parturition using vacuum tubes with a coagulation accelerator gel (BD Vacutainer®, SST II Advance, Franklin Lakes, NJ) to quantify serum glucose concentration, and vacuum tubes with a coagulation inhibitor gel (BD Vacutainer® K2, Franklin Lakes, NJ) to quantify serum levels of insulin-like growth factor 1 (IGF-1), non-esterified fatty acids (NEFA), β-hydroxybutyrate (β-OH), cholesterol, triglycerides, total protein, albumin, and urea. Both tubes were centrifuged at 2,700 × g for 20 min. Following centrifugation, the plasma and serum were collected and subsequently frozen at −20°C for further analysis. Immediately after the centrifugation, a sample of plasma was collected to assess the concentration of free amino acids (AA). In the 36th d after parturition, before the beginning of pre-induction cyclicity protocol, blood samples were collected to analyzes progesterone (P4) concentration using vacuum tubes with a coagulation inhibitor gel (BD Vacutainer® K2, Franklin Lakes, NJ). Levels of P4 higher than 1 ng/ml were considered an indicator of ovarian activity (Spitzer et al., 1995).

Serum concentrations of IGF-1 were analyzed by chemiluminescence using the Liaison analyzer and Diasorin® kit (DiaSorin, Italy). Levels of NEFA were quantified by colorimetric method, and β-OH was analyzed by kinetic enzymatic method based on oxidation of D-3-hydroxybutyrate to acetoacetate (Ref. Number FA115 and RB1007 respectively, Randox®, Ireland, United Kingdom). The concentration of free AA in

blood were obtained using the HPLC techniques described by Jones et al. (1990) and Pitta et al. (2009). Glucose (K082, Bioclin® Quibasa, Belo Horizonte, Brazil), cholesterol (K083, Bioclin® Quibasa, Belo Horizonte, Brazil), triglycerides (K117, Bioclin® Quibasa, Belo Horizonte, Brazil), and urea (K056, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by enzymatic-colorimetric method, and total protein (K031, Bioclin® Quibasa, Belo Horizonte, Brazil) and albumin (K040, Bioclin® Quibasa, Belo Horizonte, Brazil) by colorimetric method. All the analyzes previously mentioned above were determined by automated biochemical analyzer (Mindray® BS 200E, Shenzhen, China). Globulins were calculated by subtracting the albumin quantified from the total protein level.

Serum P4 concentrations was analized by the chemiluminescent method using Access Progesterone Reagent (33550, Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Hepatic tissue and skeletal muscle biopsy

Biopsies of hepatic and skeletal muscle tissues were performed only at d 27th prior to calving. In order to avoid possible abortions in an entire treatment and not impair reproductive performance in the next breeding season, six animals of each treatment were randomly selected for biopsies.

Liver sampling was performed via needle biopsy (Tru-Cut biopsy needle; Care Fusion Corporation, San Diego, CA) 4 h before supplement feeding according to the procedures described by Mølgaard et al. (2012). The incision was made between the 11th and 12th ribs for collection of samples from the right hepatic lobe (Miranda et al., 2010). Immediately, the liver samples (100 mg of tissue) were placed in cryotubes, frozen and stored in liquid nitrogen at -196°C until processing.

For the skeletal muscle, the biopsy was performed on the left side at the 13th rib three-fifths of the distance from the medial to the lateral edge of the longissimus

muscle. A 10-cm incision was made parallel to mid-point of the longissimus muscle, and the skin and fat were retracted to expose the skeletal muscle tissue. Immediately after removal of the biopsy tissue, samples (1 x 1 x 1 cm) were snap-frozen and stored in liquid nitrogen at -196°C until processing.

Abundance of carbamoyl phosphate synthase and mRNA expression of skeletal muscle energy metabolism markers

Whole liver protein was extracted in Lysis buffer (10mM Tris pH 7.2; 0,5% Triton X-100; 10% Glycerol; 0.5% Dithiothreitol; 0.5mM Phenylmethanesulfonyl fluoride and 0.5mM Benzamidine). Protein content was measured through Bradford Protein Assay (Bio-Rad, Hercules, CA, USA), and an equal amount of protein was separated through 10% dodecyl sulphate-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membranes and blocked with blocking solution (3% bovine serum albumin w/v in tris buffered saline with triton-X100 solution - TBSt) for 1 h with gentle agitation at room temperature. Membranes were then incubated with the following primary antibodies against to Carbamoyl Phosphate Synthase-1 (CPS-1 no. SC-376190, Santa Cruz, Dallas, TX, USA). Primary antibody was incubated at 1:500 diluted in the blocking solution for 16 h at 4°C with gently agitation. After incubation with primary antibody, membranes were washed 3 times at room temperature with TBSt and then incubated with the appropriate horseradish peroxidase secondary antibody (goat anti-mouse) at 1:5000 dilution, for 1 h at room temperature with gentle agitation. Then, membranes were washed 3 times (5 min each) with TBSt, developed with Clarity TM ECL substrate (Bio-Rad, Hercules, CA), scanned with c-Digit Blot scanner, and analyzed with Image Studio (LI-COR Inc., Lincoln, NE). Band density of target proteins was normalized by the density of bands obtained for a load control samples that were handled and loaded under the same conditions of the remain samples.

Total RNA (1 µg) was extracted from 0.5 g of muscle tissue samples using Trizol® reagent (Invitrogen, Carlsbad, CA, USA). The RNA integrity (RIN) was evaluated by capillary electrophoresis using a RNA 6000 Nano kit and a 2100 Bioanalyser System (Agilent Techonologies, Santa Clara, CA, USA). Samples with RIN > 7.0 were treated with DNAse I, Amplification Grade (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA using the GoScript Reverse Transcription System (Promega, Madison, WI, USA). The primer sets used for quantification of mRNA levels of carnitine palmitoyltransferase 1 (CPT-1) and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α) are shown in Table 2. Quantitative polymerase-chain reaction (qPCR) was performed on a 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using GoTaq kit (Promega, Madison, WI, USA) and the following cycle parameters: 95 °C for 3 min and 40 cycles at 95 °C for 10 s and 60 °C for 30 s. The amplification efficiency ranged from 0.90 to 0.99. After amplification, a melting curve (0.01 °C/s) was used to confirm product purity. Relative gene expression data was calculated as described by Livak and Schmittgen (2001).

Reproduction traits assessment

In the breeding season cows were pre-induced cyclicity and synchronized using the followings protocols:

For pre-induce cyclicity on d 0 (thirty-six days after calving) an intravaginal device of progesterone (Tecnopec Primer®, São Paulo, Brazil) previously utilized was inserted. On d 12 the intravaginal device was removed, and cows received an intramuscular injection of 2.0 mg of estradiol benzoate (Tecnopec RIC-BE®, São Paulo, Brazil). After 24 d the end of pre-induced protocol, the protocol for fixed time artificial insemination (FTAI) was started.

On d 0 an intravaginal device of progesterone release (Tecnopec Primer®, São Paulo, Brazil) was inserted and intramuscular injection of 2.0 mg of estradiol benzoate

(Tecnopec RIC-BE®, São Paulo, Brazil) was performed. On d 8 the intravaginal device was removed, and cows received a 2.0 mL intramuscular injection of cloprosterol sodium intramuscular (MSD Saúde Animal Ciosin®, São Paulo, Brazil) and 0.5 mL estradiol cypionat (MSD Saúde Animal Ciosin®, São Paulo, Brazil). The FTAI was preformed 48 h following intravaginal device removal (d 10). Semen from 3 Nellore sires were randomly assigned to each cow. Pregnancy diagnosis was determined via trans-rectal ultrasonography by Aloka SSD 500 (3.5 MHz linear probe; Aloka Co. Ltd., Wallingford, CT) 28 d after FTAI. Conception rate was calculated considering the cows that conceived in only one FTAI.

Statistical analysis

Statistical analyses were performed using PROC MIXED in SAS 9.4 (SAS Inst., Cary, NC) and analyzed according to a completely randomized design. The groups of animals were considered the experimental units. Treatment was considered a fixed effect whereas paddock within treatment was considered a random effect, as per the following statistical model:

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

Where, Y_{ijk} is the observation taken on subject k in the experimental unit j undergoing treatment i, μ is the overall constant; T_i is the effect of treatment i (fixed effect); $G_{(i)j}$ is the effect of the group nested to the treatment i (random effect); and $\in_{(ij)k}$ is the unobservable random error associated with each observation. Contrasts were constructed in order to evaluate the effects of supplementation and frequency of supplementation. Due to the high probability of type II error, was adopted $\alpha = 0.10$.

Initial BW and BCS were used as covariate. The choice of the best (co)variance matrix was performed following the Akaike information criterions with correction. The degrees of freedom were estimated according to the Kenward-Roger method. The

variables blood IGF-1 and metabolites were evaluated as repeated measures over time (Kaps and Lamberson, 2004).

Reproductive traits, statistical analyses were performed using a chi-square test.

RESULTS

Intake and digestibility

Forage available and forage chemical composition in the prepartum and postpartum are shown in Table 3.

Variables regarding to intake ($P > 0.10$; Table 4) and total apparent digestibility ($P > 0.10$; Table 5) were not affected by frequency of supplementation on prepartum and on postpartum.

On prepartum period, a greater CP intake ($P = 0.001$), digested OM (dOM; $P = 0.065$), and CP:dOM ratio ($P = 0.002$) was observed for supplemented cows than cows from control treatment. However, no differences were observed between supplemented and non-supplemented cows for the intake of DM ($P = 0.288$), forage DM (FDM; $P = 0.245$), OM ($P = 0.255$), apNDF ($P = 0.756$), iNDF ($P = 0.311$) and digested NDF (dNDF; $P = 0.971$). Likewise, a greater CP digestibility ($P = 0.002$), OM digestibility ($P = 0.057$) and dietary dOM ($P = 0.043$) was observed for supplemented cows than for cows at the control treatment. No difference was observed between supplemented and non-supplemented animals for apNDF digestibility ($P = 0.650$).

On postpartum period, a greater CP intake ($P = 0.018$) and CP:dOM ratio ($P = 0.039$) were observed for supplemented cows than from cows at the control treatment. However, no differences were observed between supplemented and non-supplemented cows for the intake of DM ($P = 0.484$), FDM ($P = 0.487$), OM ($P = 0.454$), apNDF ($P = 0.685$), iNDF ($P = 0.871$), dOM ($P = 0.374$) and dNDF ($P = 0.587$). Regarding to the total apparent digestibility, a greater CP digestibility ($P = 0.006$) was observed for supplemented cows than for cows at the control treatment and no difference was

observed between supplemented and non-supplemented cows for OM (P = 0.241), apNDF (P = 0.546) and dietary dOM (P = 0.204).

Animal data

The frequency of supplementation did not alter (P > 0.10; Table 6) BW, adjusted BW at day of parturition (adjBW), BW after calving upon parturition (calvingBW) ADG, BCS, ribeye area (RA), fat-thickness on longissimus muscle (FAT-*Ld*) and on *Biceps femoris* muscle (FAT-*Bf*) during pre and postpartum experimental period.

At 15 d before calving the BW (P = 0.079) and ADG (P = 0.074) were higher for supplemented cows. The RA (P = 0.352), FAT-*Ld* (P = 0.199) and FAT-*Bf* (P = 0.924) were not affect by supplementation 15 d before calving.

The adjBW (P = 0.078) was higher for supplemented cows at parturition day. The calvingBW and BCS at parturition day were not affect (P > 0.10) by supplementation and frequency of supplementation.

In additional, at 105 d after calving the supplementation did not affect (P > 0.10) BW, ADG, BCS, RA, and fat-thickness. Birth BW of calves, ADG and calf BW 105 d after calving were not different (P > 0.10; Table 6) according to supplementation and frequency of supplementation.

Hormones and metabolites levels

Insulin-like growth factor-1 levels (P = 0.744; Table 7) and glucose concentration (P = 0.865; Table 7) were similar for supplemented and non-supplemented cows. Likewise, no differences (P = 0.368) was observed between daily supplemented and infrequent supplemented cows for IGF-1, however glucose concentration was greater (P < 0.001) for cows daily supplemented than cows infrequently supplemented. Furthermore, it was observed over this trial a time effect (P < 0.001; Table 7) for supplemented and non-supplemented cows where the animals had greater (P < 0.10; Figure 2) glucose concentration at calving.

An interaction between treatment and sampling time was observed for AA ($P < 0.001$; Table 7) and SUN ($P = 0.005$; Table 7). On 9 d before calving, daily supplemented cows had higher ($P < 0.10$; Figure 3a) plasma free AA than cows from infrequent supplementation treatment, followed by greater concentrations of free AA for animals infrequently supplemented than cows non-supplemented. On 27 d postpartum, free AA levels were greater ($P < 0.10$) for cows from control and infrequent treatment than cows supplemented daily. Cows receiving supplementation had higher ($P < 0.10$; Figure 3b) SUN concentrations than cows non-supplemented on the d 27 and d 9 before calving, and at calving day.

Likewise, an interaction between treatment and sampling time was observed for glucogenic AA ($P < 0.001$), ketogenic AA ($P < 0.001$), gluco/ketogenic AA ($P < 0.001$). On 9 d before the calving, cows supplemented daily had higher ($P < 0.10$; Figure 4a, 4b, 4c) glucogenic AA, ketogenic AA, gluco/ketogenic AA, followed by greater concentrations of AA for animals infrequently supplemented than cows non-supplemented. On 27 d postpartum, glucogenic AA and gluco/ketogenic AA were greater ($P < 0.10$) for cows from control and infrequent treatment than cows supplemented daily.

No effect of supplementation ($P = 0.980$) and frequency of supplementation ($P = 0.366$) were observed on the NEFA serum concentrations. Furthermore, no effect of supplementation ($P = 0.402$) and frequency of supplementation ($P = 0.207$) were observed on the β-OH serum concentrations. The NEFA and β-OH serum levels had a time effect ($P < 0.001$) during the peripartum period (Figure 5a and Figure 5b).

Supplementation did not alter the serum concentrations of total proteins ($P = 0.122$), albumin ($P = 0.406$) and globulins ($P = 0.221$). Likewise, no difference was observed in the frequency of supplementation on total protein ($P = 0.198$), albumin ($P =$

0.795) and globulins (P = 0.678). A time effect (P < 0.001) on serum concentration of total protein and globulins were observed (Figure 6a and Figure 6b).

Cholesterol (P = 0.762) and triglycerides levels (P = 0.973) were similar between supplemented cows and non-supplemented. Likewise, no differences were observed between cows daily supplemented and cows infrequently supplemented on the cholesterol (P = 0.869) and triglycerides concentrations (P = 0.398). It was observed during this trial a time effect (P < 0.001) on serum level of cholesterol and triglycerides (Figure 7a and Figure 7b).

Abundance of carbamoyl phosphate synthase and mRNA expression of skeletal muscle energy metabolism markers

No effect of supplementation (P = 0.815; Table 8) and frequency of supplementation (P = 0.987) was observed on the overall content of hepatic CPS-1 protein.

Regarding to the biomarkers associated with beta-oxidation of fatty acids, the supplementation did not alter the mRNA abundance of PGC-1α (P = 0.433) and CPT-1 (P = 0.273). Likewise, the supplementation frequency did not alter the mRNA abundance of PGC-1α (P = 0.365) and CPT-1 (P = 0.164).

Reproduction traits

Percentage of cows with luteal activity (P4 > 1 ng/ml) was not different (P = 0.606; Table 9) between the treatments. Likewise, no difference was observed for conception rate (P = 0.842).

DISCUSSION

In grazing cattle livestock system, the quantity of forage available can affect feed intake (NASEM, 2016). Thus, applying the concept of pdDM that integrates the forage availability and forage quality regardless of season, the overall average pdDM

mass at prepartum (81 g/kg BW) and at postpartum period (89 g/kg BW) were higher than 40 to 50 g/kg BW for satisfactory intake and performance in a grazing system (Paulino et al., 2004). Therefore, the quantity of forage available in this trial can allowance to animals the possibility of selective grazing and it is considered non-restrictive for feed intake (Paulino et al., 2004).

The adequacy of dietary protein-to-energy ratio has been pointed out as one of the main indicators of the intake pattern of cattle fed tropical forages (Detmann et al., 2014b). The maximum forage intake is observed with dietary CP:dOM approximately at 216 g/kg (Reis et al., 2016). Despite of higher dietary CP:dOM for supplemented cows (Table 4) than non-supplemented cows at pre and postpartum period, the dietary CP:dOM observed in our study for cows daily supplemented and cows infrequently supplemented were below the value of CP:dOM suggested by Reis et al. (2016). Therefore, all treatments had unbalanced dietary protein-to-energy ratio (CP deficiency) when adequacy of intake is considered, which seems to support the unaltered forage intake among supplemented and non-supplemented cows, and between the cows from different supplementation frequency. Furthermore, according to Detmann et al. (2014b), the voluntary intake of forage has been stimulated with dietary CP levels close to 145 g/kg DM. Nevertheless, although the supplementation to fail to increase the forage voluntary intake, the similar FDM among cows supplemented daily and cows supplemented every three days is a possible indicative that the decreasing of the frequency of supplementation can be attractive to cow-calf producers. Usually, infrequent supplementation under *medium-high* quality forage has been reported by a decreasing in the forage voluntary intake (Loy et al., 2007; Rufino, 2015).

Supplementation increased CP intake (Table 4) at pre and postpartum period due to the additional supply of protein through of the supplement. Moreover, at prepartum period, there was higher digestibility of OM and CP (Table 5), providing higher dOM

for supplemented animals than non- supplemented animals (Table 4). Such a pattern has been associated with the inclusion of supplement in the diet of the animals, since concentrates usually have higher digestibility than forage (Paulino et al., 2008b), and provide lower proportion of metabolic fecal fraction in relation to ingested nutrients (Van Soest, 1994; Barros et al., 2011). However, at postpartum period the supplementation increased only the CP digestibility (Table 5). Since was offered a same amount of equivalent mass by day of supplement to cows supplemented every three days, associated with an unaltered forage intake among daily supplemented and infrequently supplemented cows, may suggest that this is the reason for no difference for intake of CP and OM at prepartum period and the intake of CP at postpartum period among the different treatments of supplementation frequency.

The increase of dOM and CP intake increased the BW, adjBW and ADG (Table 6) at prepartum period for supplemented cows. Although, primiparous cows seem to be more sensitive to nutrient intake and consequently BCS changes (NASEM, 2016), such a pattern it was not observed in this trial at prepartum period. Furthermore, the BCS at calving for all treatments was between the minimum (5.0) and maximum (6.0) recommended by the NASEM (2016) so that females have reproductive success in the breeding season. These data suggest that cows (control treatment) can adapt energy metabolism (e.g. maintenance energy) to periods of lower availability of nutrients (Blanc et al., 2006; Mulliniks et al., 2016), resulting similar (Table 6) BCS, ribeye area and fat-thickness compared to supplemented cows as evidenced in the prepartum period. By the other way, the absence of effect on intake of dOM at postpartum period resulted similar performance for supplemented and non-supplemented cows. Similar pattern was observed between the cows from of different supplementation frequency (Table 6). Thus, since was not observed difference between cows supplemented daily and cows supplemented every three days regarding to BW, adjBW, calvingBW, ADG, BCS, RA,

FAT-*Ld* and FAT-*Bf*, these data may suggest that the providing of supplement for cows every three days is a viable alternative in cow-calf production system.

Although maternal intake during the last trimester of pregnancy has been reported an important factor for fetal growth (NASEM, 2016) altering birth weight of progeny (Spitzer et al., 1995; Radunz et al., 2010), no difference was observed for calf birth BW between supplemented and non-supplemented cows (Table 6). The forage quality has been pointed out as one of the main reasons for this result (Silva, 2016). Furthermore, a greater nutritional plane in primiparous suckled beef has been associated with greater calf BW and calf ADG (Astessiano et al., 2012). However, in our study, the nutritional strategies applied to dams did not improve calves' performance (Table 6). The absence of effect on intake of dOM and similar cows' body reserves between supplemented and non-supplemented cows during postpartum period, probably reflected on similar milk yield between supplemented and non-supplemented cows providing similar calves' performance (Table 6).

Usually, periods of negative energy balance (NEB) occur during the last months of pregnancy and initial lactation in beef cows (Laporta et al., 2014). Thus, metabolic homeostasis to support pregnancy and/or milk production is need. Low levels of IGF-I, elevate serum concentrations of GH and decrease insulin concentrations during NEB stimulating hepatic gluconeogenesis to provide glucose for the fetus or for lactose synthesis. Furthermore, NEFA is mobilized from adipose tissue to provide energy for peripheral needs (Bauman, 2000). Consequently, elevated levels of NEFA may result in ketone bodies production, such as β-OH, another energetic substrate (Mulliniks et al., 2013). Thus, we suggest that the intake of nutrients to support pregnancy and/or milk production during the peripartum period was enough to avoid problems concern energy balance between supplemented and non-supplemented cows. The absence of effects (Table 7) on NEFA and β-OH among supplemented and non-supplemented cows

support such argument. The same pattern was observed among cows supplemented daily and cows supplemented every three days. Furthermore, serum NEFA concentrations during transition period (Table 7; Figure 5a) was below the threshold of 0.40 mmol/L, value suggested by Oetzel (2004) as an indicative of problems concerning energy balance in dairy cattle, and same value utilized by Lopes et al. (2016) in beef cattle.

Levels of cholesterol and triglycerides among supplemented and non-supplemented cows, and between the studied frequency treatments were not different (Table 7). These data may suggest that all treatments were able to cope with the β-oxidation of NEFA and export those not used as metabolic fuel, which resulted in similar serum triglycerides and cholesterol levels among supplemented and non-supplemented cows, and between the studied frequency treatments.

The dietary intake of starch substrates is directly associated with greater hepatic gluconeogenesis in ruminants (Drackley et al., 2001). Nevertheless, the decreasing of supplementation frequency decreased plasma concentrations of glucose (Table 7). Probably, since blood sampled only at one single day, after 3 days since infrequent supplementation and before the next infrequent supplementation, the plasma glucose concentration was decreasing through of the days that no supplementation was offered. Furthermore, since was not observed difference between cows daily supplemented and cows from infrequent treatment in the levels of metabolites derived from fatty acid metabolism (NEFA and β-OH) to meet the glucose metabolic requirements, these data support such argument that glucose difference between the treatments of different frequency of supplementation is due to supply daily of substrates associated hepatic gluconeogenesis. However, the lack of difference on plasma concentration of glucose among supplemented and non-supplemented cows, may suggest that is due the large glucose levels dispersion between cows of different supplementation frequency

(difference among the frequency of supplementation), that this pattern resulted in similar levels of glucose compared to control treatment when the orthogonal contrast was constructed.

When energy demands exceed energy intake, body fat mobilization increases the expression of genes involved in fatty acid oxidation and utilization, such as CPT-1 and PGC-1α (Brennan et al., 2009; Wood et al., 2013). Therefore, similar NEFA levels, among supplemented and non-supplemented cows and, between cows supplemented daily and cows supplemented every three days, may suggest that is the main reason for absence of effect (Table 8) on CPT-1 and PGC-1α mRNA expression among, supplemented and non-supplemented cows and, between cows supplemented daily and cows supplemented every three days. This pattern is another indicative that may suggest no problems concerning energy balance according to supplementation and supplementation frequency in our study.

The dependency of nitrogen recycling to the rumen is other indicative of dietary protein deficiency (Detmann et al., 2014a; Detmann et al., 2014b). Data reported by Batista (2017a) and Batista (2017b), on tropical forage of *medium-high* quality, suggest a reduction on SUN due its transfer to the rumen to keep microbial growth. Furthermore, under the previous conditions, without the myofibrillar protein mobilization to sustain the nitrogen recycling (Rufino et al., 2016; Batista et al., 2017a), associated to a predominant use of absorbed AA for nitrogen recycling, may suggest a low peripheral circulation pool of AA. Thus, on the d 27 and d 9 before calving, and at calving day, the supplemented animals had higher SUN levels (Figure 3b) than cows from control treatment due the increase in dietary nitrogen intake is associated with larger ammonia transfer of rumen to into the blood, and a larger output of urea from the liver (Van Soest, 1994), besides a lower NUS transfer to the rumen to keep microbial growth. However, the *pool* of AA was higher only at ninth day before calving (Figure

3a) for supplemented animals. Probably, only at this day, supplemented cows had enough escape of dietary protein to avoid the use of metabolizable AA to synthesis of urea for recycling.

The lactation peak in Nellore cows occurs between the third and postpartum fouth week (Costa e Silva et al., 2016). Thus, the higher blood AA for cows from control and infrequent treatment than cows from daily treatment at day 27 after parturition, may suggest that occurred in order to support milk production (milk protein or lactose production), since these treatments had not a daily constant availability of energy and protein compounds through of supplement. The larger amount of glucogenic AA (Figure 4a), a precursor of lactose, for cows from control and infrequent treatment at day 27 after parturition are corroborated with the previous knowledge above. By the other way, since the blood sampling occurred on the third day of supplementation cycle, may suggest that the AA mobilization was increasing through the days that no supplementation was offered for cows from infrequent treatment compared to cows from daily treatment. Furthermore, the patterns of levels of glucogenic AA, ketogenic AA, and gluco/ketogenic AA (Figure 4c) among all the treatments, at nine days before calving and at twenty-seven postpartum days, are due to the control homeostatic of AA concentration (Van Soest et al., 1994) corroborating with the free AA pool pattern of behavior (Figure 3a) in the blood.

Protein dietary intake (severe restriction or excess protein intake) and the protein supply on infrequent days have been reported with a greater action of enzymes involved in the urea cycle, such as CPS-1 (Cappellozza et al., 2015; Sun et al., 2016). The CP intake (Table 4) of cows non-supplemented was above to the level minimum required to meet the maintenance requirement (BR-CORTE, 2016). Furthermore, cows daily supplemented had the CP intake below of threshold maximum that positive responses to supplemental protein has been observed (225 g CP/kg DM; Detmann et al., 2014b).

Thus, may suggest that the main reason for absence of difference in the hepatic CPS-1 abundance (Table 8) between supplemented and non-supplemented cows is due to protein dietary intake was not low or excess to alter abundance of CPS-1. We suggest that the similar CPS-1 abundance among cows daily supplemented and cows supplemented every three days can be explained the previous knowledge above, since that in the day of infrequent supplementation, the CP intake was approximately of 200 g CP/kg DM (no excess protein intake).

Levels of serum total protein and albumin reflect the proteic *status* at long-term due low variability in blood (Ndlovu et al., 2007). Furthermore, deficiency of protein can alter humoral immunity (Titgemeyer and Löest, 2001), altering serum globulins concentration. In our study, no difference was observed between supplemented and non-supplemented cows on serum concentration of total protein, albumin and globulins (Table 7). Since was not observed a severe deficiency of dietary CP intake for non-supplemented cows according to BR-CORTE (2016), may suggest this is the main reason for similar results of serum total protein, albumin and globulins between supplemented and non-supplemented cows. Likewise, we suggest that this is the same reason explain the similar results among cows of different frequency of supplementation. Furthermore, cows supplemented every three days did not have a long period without protein supplementation.

Among the main effects controlling postpartum anestrus, energy dietary intake and body condition score at calving have been reported (Short et al., 1990; NASEM, 2016). Thus, since that was observed similar intake of dietary energy on postpartum period and similar body reserves throughout the experimental period (BCS and fat-thickness), these data may be the main reasons that led to a similar percentage (Table 9) of cows with luteal activity and conception rate among all the treatments.

CONCLUSION

The energy-protein supplementation during pre and postpartum of primiparous beef cows under grazing conditions do not improve performance and metabolic characteristics. However, the reduction of frequency of supplementation do not result negative effects on performance and metabolic characteristics.

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TABLES AND FIGURES

Table 1. Feeds and chemical composition of experimental supplement

Item	Supplement
Ingredients (g/kg; as-fed basis)	
Wheat meal	425
Corn meal	213
Soybean meal	332
Urea	27
Ammonium sulfate	3
Mineral mixture[1]	*Ad libitum*
Chemical composition (g/kg; dry matter basis)	
Organic matter	955
Crude protein	340
Total digestible nutrients	838
apNDF[2]	186

[1]87 g/kg of calcium ($CaCO_3$), 90 g/kg of phosphor ($CaHPO_4$), 187 g/kg sodium (NaCl), 90 g/kg sulfur ($CuSO_4.(H_2O)_5$), 2400 mg/kg of zinc ($ZnSO_4$), 800 mg/kg of copper ($CuSO_4.(H_2O)_5$), 1600 mg/kg of manganese ($MnSO_4$.H2O), 40 mg/kg of iodine (KIO_3), 8 mg/kg of cobalt ($CoSO_4.(H2O)_7$), 8.16 mg/kg of selenium ($Na_2SeO_3.(H_2O)_5$).

[2]apNDF neutral detergent fiber assayed with a heat stable amylase and corrected of residual ash and protein.

Table 2. Primer sequences for all genes transcripts analyzed by quantitative real-time reverse transcription PCR

Gene[1]	Primer sequence	UniGene access code
CPT-1		NP_001079706.1
Forward	5'-GTCCCTTCCCTTGCTCTA-3'	
Reverse	5'-GGACAGCAGAGACCCATA-3'	
PGC-1α		NP_808814.1
Forward	5'-GAAGCGGGAATCCGAAAG-3'	
Reverse	5'-CTCAGTTCTGTCCGTGTTG-3'	
18S		NM_001033614
Forward	5'-CCTGCGGCTTAATTTGACTC-3'	
Reverse	5'-AACTAAGAACGGCCATGCAC-3'	

[1]CPT-1 carnitine palmitoyltransferase 1; PGC-1α peroxisome proliferator-activated receptor gamma coactivator 1α; 18S eukaryotic ribosomal RNA.

Table 3. Potentially digestible forage mass and chemical forage composition during pre and postpartum of grazing primiparous beef cows

Item[1,2]	Prepartum		Postpartum		*Brachiaria decumbens*[6]
	Brachiaria decumbens[4]	*Brachiaria decumbens* (Intake trial)[5]	*Brachiaria decumbens*[4]	*Brachiaria decumbens* (Intake trial)[5]	
pdDM[3]	2560±328	2346±205	2518±186	2807±362	2539±331
OM	918±1.3	919±1.0	924±2.1	929±1.6	921±1.4
CP	69±2.2	73±1.7	73±2.8	68±3.1	71±1.8
NIDIN	185±9.1	282±10.5	155±7.9	272±4.8	171±7.1
apNDF	586±14.1	546±9.3	564±6.9	568±12.9	576±8.4
iNDF	213±10.6	180±4.1	223±14.3	210±5.7	218±8.5

[1]pdDM potentially digestible forage dry matter, kg/ha; OM organic matter, g/kg; CP crude protein, g/kg; NIDIN neutral detergent insoluble N, g/kg of total N; apNDF neutral detergent fiber corrected for ash and protein residue, g/kg; iNDF indigestible neutral detergent fiber, g/kg.
[2]Chemical composition was evaluated in the hand-plucked forage sample.
[3]pdDM was estimated for forage sampled in the area delimeted by a metal square 0.5x0.5m.
[4]Mean ± standard error of the mean of pdDM and chemical composition of forage during the pre and postpartum period.
[5]Mean ± standard error of the mean of pdDM and chemical composition of forage during the pre and postpartum intake trial.
[6]Mean ± standard error of the mean of the mean of pdDM and chemical composition of forage during the experimental period.

Table 4. Intake according to frequency of supplementation during pre and postpartum of grazing primiparous beef cows

Item[1]	Treatment			SEM[2]	*P* value[3]	
	Control	Daily	Infrequent		C *vs.* S	D *vs.* I
Prepartum						
		kg/d				
DM	7.69	8.46	8.17	0.394	0.288	0.644
FDM	7.69	7.13	6.84	0.394	0.245	0.644
OM	7.07	7.82	7.57	0.361	0.255	0.678
CP	0.52	0.98	0.98	0.030	0.001	0.988
apNDF	4.19	4.14	4.03	0.247	0.756	0.782
iNDF	1.41	1.35	1.27	0.070	0.311	0.478
dOM	4.41	5.32	5.20	0.242	0.065	0.753
dNDF	2.69	2.72	2.64	0.233	0.971	0.824
		g/kg				
CP:dOM	119	184	188	5.1	0.002	0.607
		Intake (g/kg of BW)				
DM	17.9	19.7	19.2	0.95	0.266	0.720
Forage DM	17.9	16.6	16.1	0.94	0.280	0.709
OM	16.4	18.2	17.8	0.87	0.237	0.761
apNDF	9.7	9.7	9.5	0.60	0.833	0.835
iNDF	3.3	3.1	3.0	0.17	0.365	0.543
Postpartum						
		kg/d				
DM	8.49	9.15	9.17	0.682	0.484	0.985
FDM	8.49	7.82	7.84	0.682	0.487	0.985
OM	7.89	8.56	8.52	0.616	0.454	0.961
CP	0.58	0.95	1.03	0.072	0.018	0.492
apNDF	4.84	4.74	4.61	0.307	0.685	0.783
iNDF	1.73	1.81	1.63	0.075	0.871	0.211
dOM	4.20	4.67	4.98	0.490	0.374	0.688
dNDF	2.80	2.56	2.66	0.259	0.587	0.797
		g/kg				
CP:dOM	137	205	211	16.5	0.039	0.814
		Intake (g/kg of BW)				
DM	19.9	21.0	21.8	1.64	0.516	0.777
Forage DM	19.9	17.9	18.6	1.61	0.471	0.792
OM	18.5	19.7	20.2	1.49	0.486	0.819
apNDF	11.4	10.9	11.0	0.82	0.672	0.952
iNDF	4.1	4.1	3.9	0.17	0.683	0.351

[1]DM dry matter, FDM forage dry matter, OM organic matter, CP crude protein, apNDF neutral detergent fiber assayed with a heat stable amylase and corrected for residual ash and protein, iNDF indigestible neutral detergent fiber, dOM digested organic matter, dNDF digested neutral detergent fiber.

[2]Standard error of the mean.

[3]C *vs.* S = contrast between supplemented and non-supplemented cows; D *vs.* I = contrast between supplemented cows daily and supplemented cows every three days.

Table 5. Total apparent digestibility and dietary concentration of digested organic matter according to frequency of supplementation during pre and postpartum of grazing primiparous beef cows

Item[1]	Treatment			SEM[2]	*P* value[3]	
	Control	Daily	Infrequent		C *vs.* S	D *vs.* I
Prepartum						
		g/g				
OM	0.626	0.684	0.688	0.1616	0.057	0.887
CP	0.450	0.693	0.700	0.0175	0.002	0.785
apNDF	0.642	0.657	0.653	0.0210	0.650	0.895
		g/kg dry matter				
dOM	575	632	638	14.3	0.043	0.788
Postpartum						
		g/g				
OM	0.531	0.547	0.581	0.0187	0.241	0.301
CP	0.318	0.499	0.568	0.0254	0.006	0.155
apNDF	0.578	0.541	0.575	0.0247	0.546	0.401
		g/kg dry matter				
dOM	493	512	540	16.6	0.204	0.335

[1]OM organic matter, CP crude protein, apNDF neutral detergent fiber assayed with a heat stable amylase and corrected for residual ash and protein, dOM digested organic matter.
[2]Standard error of the mean.
[3]C *vs.* S = contrast between supplemented and non-supplemented cows; D *vs.* I = contrast between supplemented cows daily and supplemented cows every three days.

Table 6. Performance according to frequency of supplementation during pre and postpartum of grazing primiparous beef cows

Item[1]	Treatment			SEM[5]	*P* value[6]	
	Control	Daily	Infrequent		C *vs.* S	D *vs.* I
Prepartum						
105 d before calving						
BW (kg)	411	408	409	14.5	0.912	0.982
BCS (1-9)	5.5	5.4	5.5	0.23	0.932	0.831
RA (cm^2)	48.9	51.7	49.1	2.36	0.650	0.501
FAT-*Ld* (mm)	2.5	3.6	3.8	0.72	0.267	0.812
FAT-*Bf* (mm)	4.2	6.0	6.1	1.16	0.279	0.923
15 d before calving						
BW (kg)	436	451	443	3.3	0.079	0.184
ADG[2] (kg/d)	0.35	0.54	0.43	0.042	0.074	0.180
RA (cm^2)	51.0	49.2	51.0	0.60	0.352	0.133
FAT-*Ld* (mm)	3.2	3.8	3.5	0.17	0.199	0.362
FAT-*Bf* (mm)	5.7	5.9	5.4	0.16	0.924	0.146
Parturition						
adjBW[3] (kg)	451	475	460	5.0	0.078	0.118
calvingBW(kg)	403	423	411	6.9	0.205	0.296
BCS (1-9)	5.7	6.0	6.0	0.20	0.251	0.946
calfBW (kg)	30	31	30	1.6	0.923	0.738
Postpartum						
105 d after calving						
BW (kg)	436	441	442	12.0	0.757	0.969
ADG[4] (kg/d)	0.32	0.15	0.31	0.084	0.430	0.275
BCS (1-9)	5.3	5.5	5.6	0.19	0.297	0.761
RA (cm^2)	51.5	49.6	52.7	1.67	0.894	0.300
FAT-*Ld* (mm)	3.2	4.0	3.9	0.57	0.452	0.944
FAT-*Bf* (mm)	5.6	6.0	4.8	0.59	0.806	0.235
calfBW (kg)	119	124	122	3.9	0.538	0.759
calfADG (kg/d)	0.85	0.89	0.88	0.037	0.552	0.779

[1]BW body weight, adjBW adjusted BW for day of parturition, calvingBW BW after calving upon parturition, ADG average daily gain, BCS body condition score, RA ribeye area, FAT-*Ld* backfat-thickness on longissimus muscle, FAT-*Bf* backfat-thickness on *Biceps femoris* muscle, calfBW calf body weight, calfADG calf average daily gain.

[2]$ADG_{prepartum} = [(BW_{15\ d\ before\ calving} - BW_{105\ d\ before\ calving}) \div 90\ days]$

[3]$adjBW = [BW_{15\ d\ before\ calving} + (ADG_{prepartum} \times number\ of\ days\ until\ parturition)]$

[4]$ADG_{postpartum} = [(calvingBW - BW_{105\ d\ after\ calving}) \div 105\ days]$

[5]Standard error of the mean.

[6]C *vs.* S = contrast between supplemented and non-supplemented cows; D *vs.* I = contrast between supplemented cows daily and supplemented cows every three days.

Table 7. Endocrine and metabolic profile according to frequency of supplementation during pre and postpartum of grazing primiparous beef cows

Item	Treatment			SEM[2]	*P* value[3]			
	Control	Daily	Infrequent		C *vs.* S	D *vs.* I	T	T x Treat
Insulin-like growth factor-1 (ng/mL)	206	222	202	16.4	0.744	0.368	0.312	0.413
Glucose (mg/dL)	69.2	72.3	65.6	0.97	0.865	<0.001	<0.001	0.328
Free amino acids (nmol/mL)	1689	1717	1712	51.4	0.665	0.938	<0.001	<0.001
Glucogenic amino acids (nmol/mL)	1563	1576	1563	46.1	0.896	0.826	<0.001	<0.001
Ketogenic amino acids (nmol/mL)	127	145	150	7.7	0.115	0.671	<0.001	<0.001
Gluco/ketogenic amino acids (nmol/mL)	292	305	306	12.8	0.406	0.928	<0.001	<0.001
SUN[1] (mg/dL)	10.6	17.3	13.9	1.11	0.034	0.109	<0.001	0.005
Non-esterified fatty acids (mmol/L)	0.21	0.23	0.18	0.026	0.980	0.366	<0.001	0.622
β-hydroxybutyrate (mmol/L)	0.45	0.52	0.45	0.035	0.402	0.207	<0.001	0.639
Total protein (g/dL)	6.58	6.74	6.64	0.127	0.122	0.198	0.006	0.809
Albumin (g/dL)	3.38	3.31	3.28	0.077	0.406	0.795	0.234	0.879
Globulins (g/dL)	3.19	3.43	3.36	0.166	0.221	0.678	0.001	0.827
Cholesterol (mg/dL)	125.7	128.2	130.5	10.20	0.762	0.869	<0.001	0.958
Triglycerides (mg/dL)	26.6	27.8	25.2	1.92	0.973	0.398	<0.001	0.191

[1]SUN Serum urea nitrogen was estimated as 46.7% of the total serum urea.
[2]Standard error of the mean.
[3]C *vs.* S = contrast between supplemented and non-supplemented cows; D *vs.* I = contrast between supplemented cows daily and supplemented cows every three days; T = Time, days relative at calving; T x Treat = interaction between sampling time and treatment.

Table 8. Abundance of hepatic protein associated with ureagenesis (CPS-1) and expression of mRNA markers associated with beta oxidation of non-esterified fatty acids (CPT-1 and PGC-1α) in skeletal muscle according to frequency of supplementation during prepartum of grazing primiparous beef cows

Item[1] (arbitrarity units)	Treatment			SEM[2]	*P* value[3]	
	Control	Daily	Infrequent		C *vs.* S	D *vs.* I
CPS-1	0.86	0.81	0.81	0.133	0.815	0.987
CPT-1	8.88	6.97	8.67	0.639	0.273	0.164
PGC-1α	7.03	5.36	6.71	0.903	0.433	0.365

[1]CPS-1 carbamoyl phosphate synthetase I; CPT-1 carnitine palmitoyltransferase 1; PGC-1α peroxisome proliferator-activated receptor γ coactivator 1α.
[2]Standard error of the mean.
[3]C *vs.* S = contrast between supplemented and non-supplemented cows; D *vs.* I = contrast between supplemented cows daily and supplemented cows every three days.

Table 9. Parameters of reproduction according to frequency of supplementation during pre and postpartum of grazing primiparous beef cows

Item	Treatment			*P* value[1]
	Control	Daily	Infrequent	
	%			
Animals eligible for breeding (ovarian activity = P4 > 1 ng/ml)	37.5	50.0	62.5	0.606
Conception rate	37.5	37.5	50.0	0.842

[1]*P* value > 0.10 = Proportions on the same line do not differ statistically by the chi-square test.

1

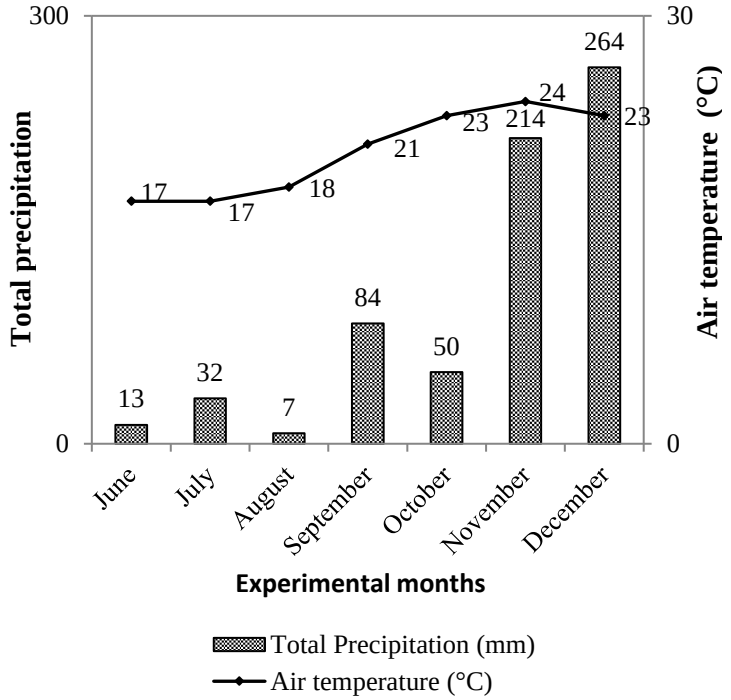

Figure 1. Total precipitation and average air temperature from June to December 2015. Source: DEA/UFV

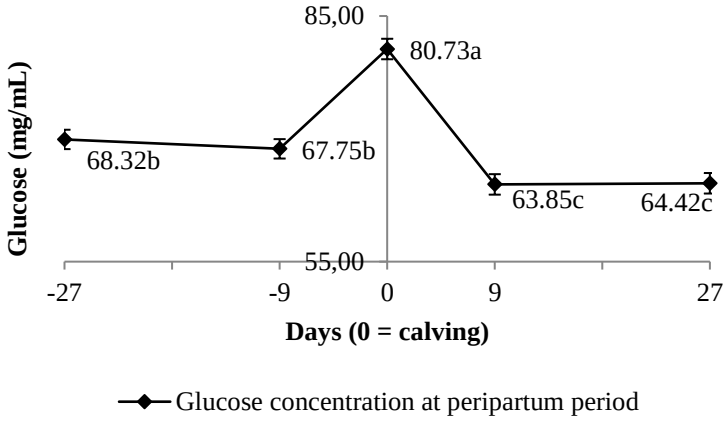

Figure 2. Pattern of glucose concentration during pre and postpartum of grazing primiparous beef cows. Least square means followed by different letters are different ($P < 0.10$).

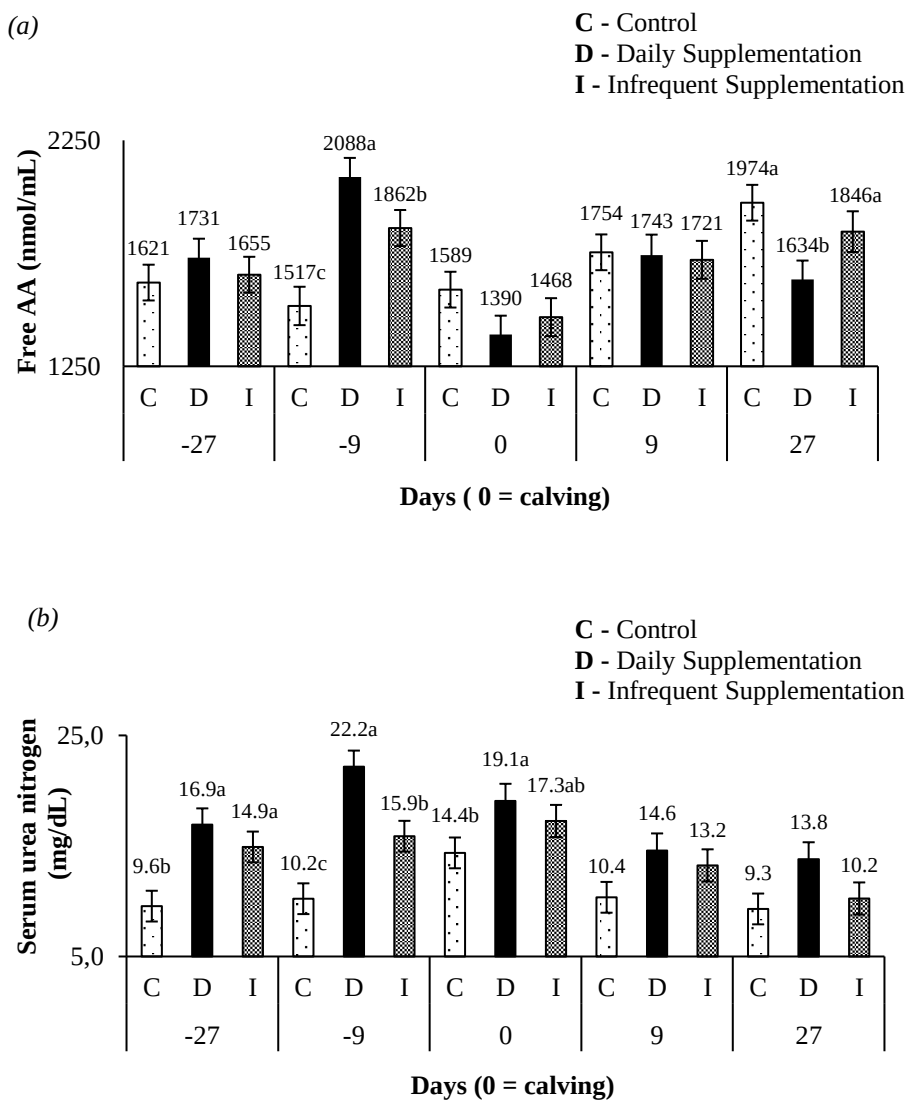

Figure 3. Serum concentration of (a) free amino acids and (b) serum urea nitrogen as a function of the days of sampling and the different treatments. Least square means within the sampling days followed by different letters are different ($P < 0.10$).

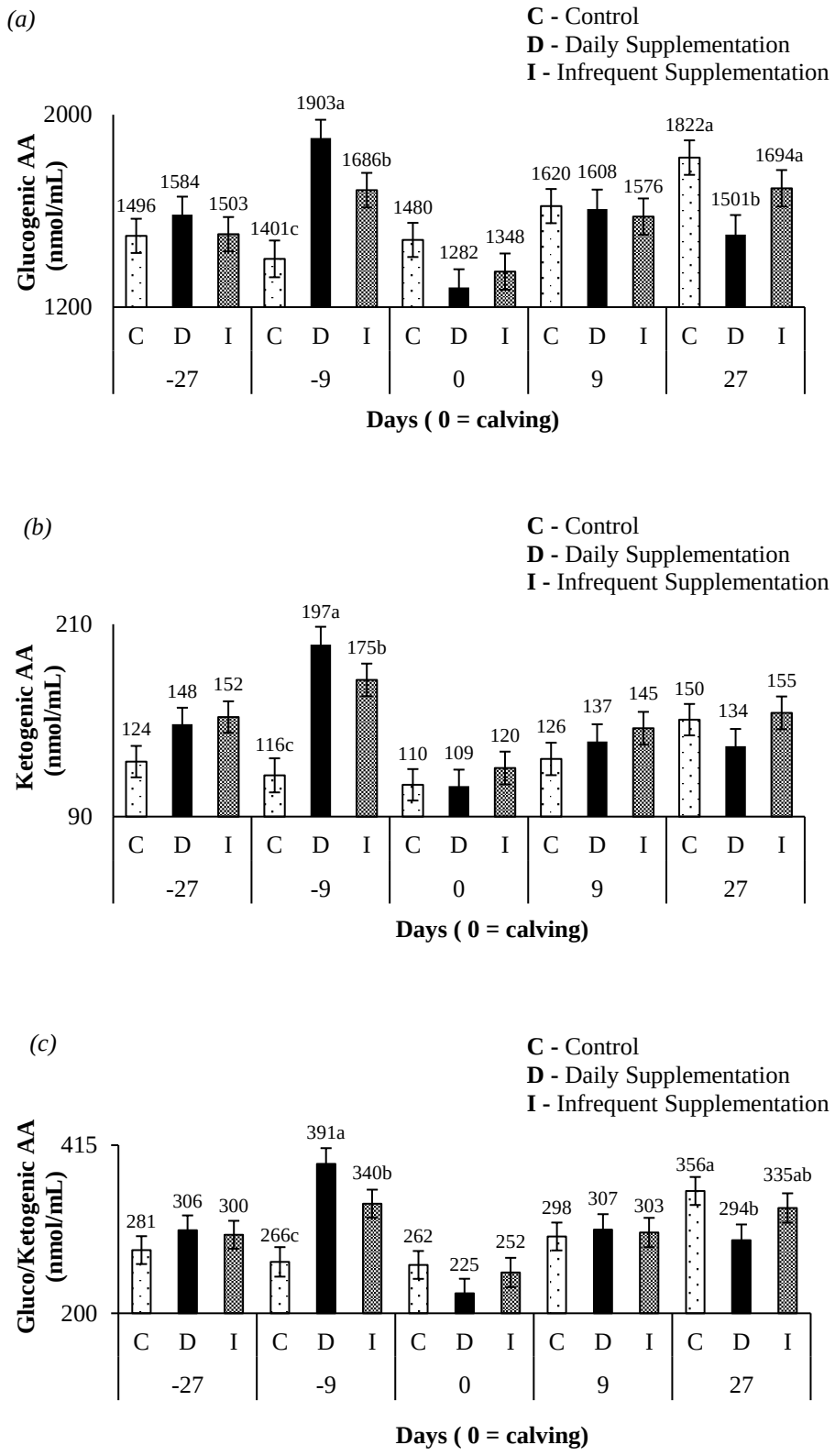

Figure 4. Serum concentration of (a) glucogenic amino acids, (b) ketogenic amino acids and (c) gluco/ketogenic amino acids as a function of the days of sampling and the different treatments. Least square means within the sampling days followed by different letters are different ($P < 0.10$).

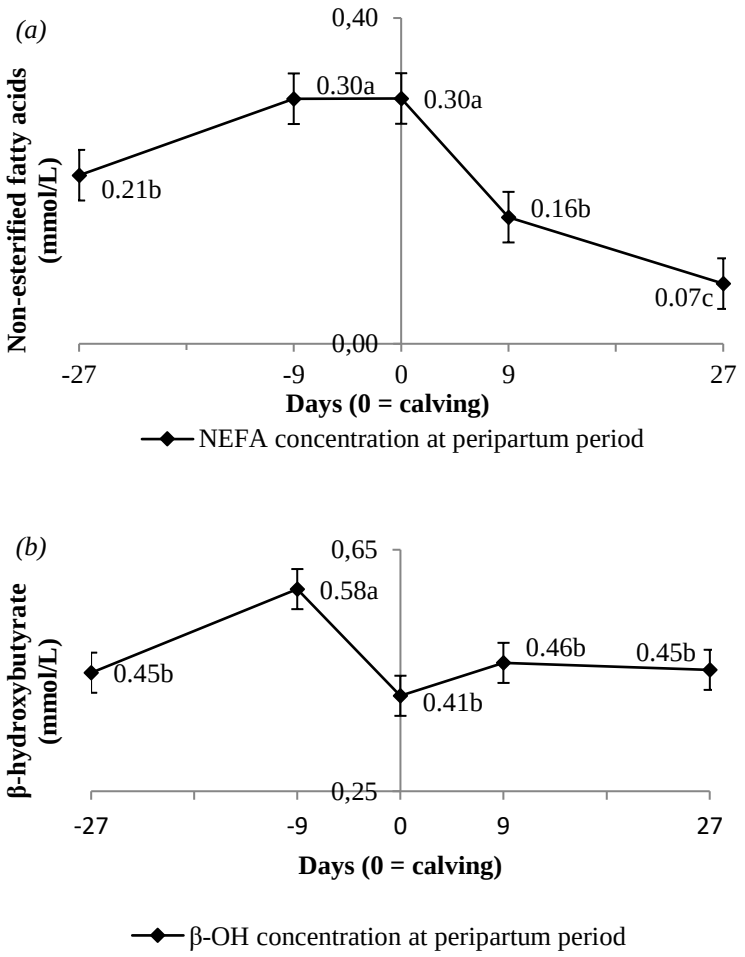

Figure 5. Pattern of (a) non-esterified fatty acids concentration and (b) β-hydroxybutyrate during pre and postpartum of grazing primiparous beef cows. Least square means followed by different letters are different ($P < 0.10$).

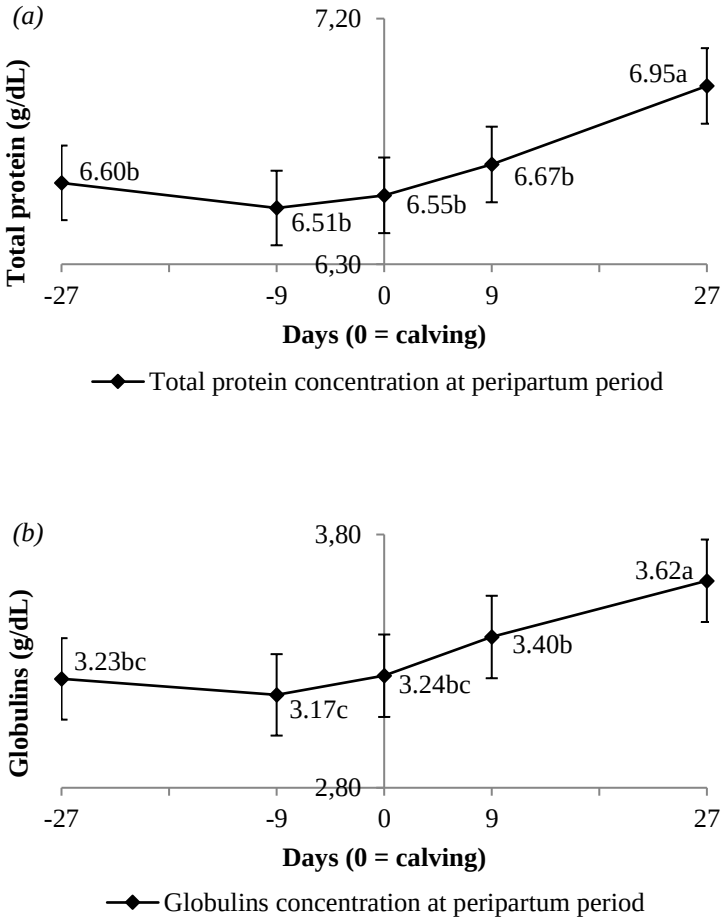

Figure 6. Pattern of (a) total protein and (b) globulins concentration during pre and postpartum of grazing primiparous beef cows. Least square means followed by different letters are different ($P < 0.10$).

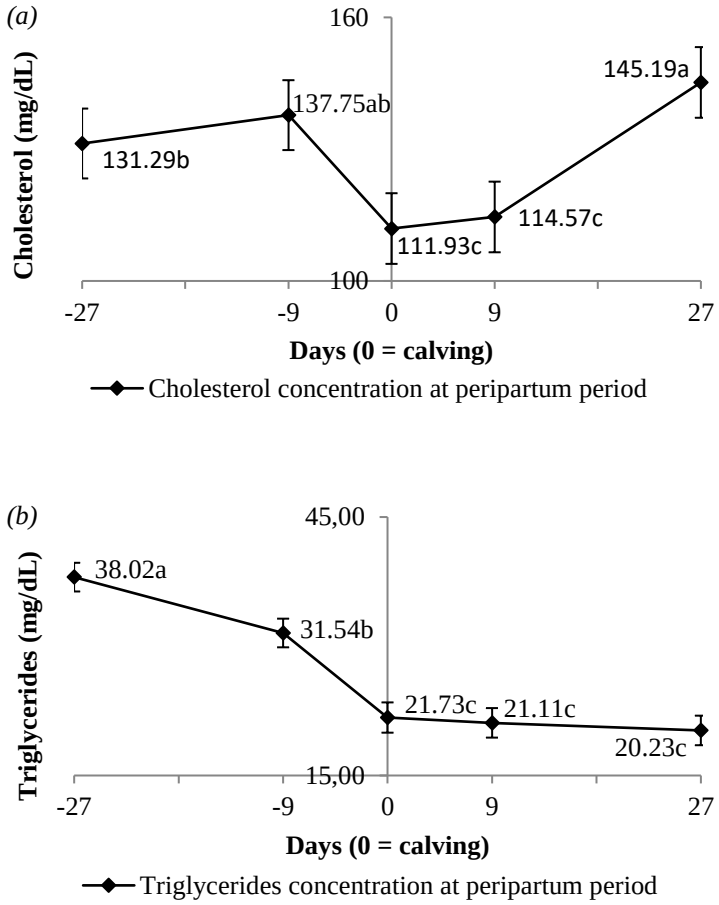

Figure 7. Pattern of (a) cholesterol and (b) triglycerides concentration during pre and postpartum of grazing primiparous beef cows. Least square means followed by different letters are different ($P < 0.10$).