MATHEUS FELLIPE DE LANA FERREIRA

PERFORMANCE, METABOLIC AND HORMONAL RESPONSES ON PERIPARTUM OF GRAZING BEEF CATTLE SUPPLEMENTED ON PRE-PARTUM

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*.

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To my parents Willian and Roseli, my brothers Natália, Otávio and Caio, and my girlfriend Thalita for the encouragement, love, and unconditional support. Without you, I'm nothing.

I dedicate.

"…*But I feel I'm growing older, and the songs that I have sung echo in the distance like the sound of a windmill going round guess I'll always be a soldier of fortune*"

Deep purple

(Coverdale/Blackmore)

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Thank you, so much!

BIOGRAPHY

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

He 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.S. program with major on physiologic and ruminant production.

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ABSTRACT

FERREIRA, Matheus Fellipe de Lana, M.Sc., Universidade Federal de Viçosa, February, 2018. **Performance, metabolic and hormonal responses on peripartum of grazing beef cattle supplemented on pre-partum**. Adviser: Luciana Navajas Rennó. Co-advisers: Edenio Detmann and Mário Fonseca Paulino.

Nutritional status at calving is the main factor affecting time to pregnancy. Metabolic parameters that relate nutritional status to physiological processes within grazing animals, mainly Zebu, are not fully understood. This study evaluated the effects of 60-day pre-partum energetic-protein supplementation on performance, metabolic and hormonal responses on peripartum of grazing beef cattle. Thirty-eight Nellore multiparous cows averaging 230 ± 10 days of gestation were used. Two treatments were evaluated: control, without supplementation and daily supplementation (30% of CP) with 1.5 kg during the 60 days before expected calving. The experiment was carried out according to completely randomized design. It was calculated the average daily gain pre-calving and post-calving. The calves were weighted at 45 and 90 days. Body condition scores (BCS) were also recorded at the beginning of the experiment, at calving and 45 days post-partum. Every 30 days grass samples were collected by hand plucked sampling and collected cutting at ground level. To evaluate intake and digestibility, a trial was performed at 45 days before the estimated date of parturition. It was used titanium dioxide (TiO2) to estimate the fecal excretion of animals and indigestible neutral detergent fiber (iNDF) to estimate the pasture dry matter intake (DMI). It was assumed that the supplement consumption will be equal to the quantity offered per animal/day. At last day of the trial, spot urine samples were collected 4 hours before and after the supplement offer. At the 30 and 45 days post-calving, were performed milking to estimate the milk production. Taking calving day as day 0, blood samples were collected before supplementation on days -30, 0, 15, 30, 45 by jugular vein puncture, using vacuum tubes to quantity glucose, blood nitrogen urea (BUN), total protein, albumin, triglycerides, total cholesterol, high density lipoprotein (HDL), non-esterified fatty acid (NEFA), beta-hydroxybutyrate (βHB), insulin, insulin-like growth factor (IGF-1), total triiodothyronine (T3), total thyroxine (T4) and progesterone (P4) contents. It was calculated the pregnancy rate and number of

days from calving to conception. ADG was higher at pre-partum (P<0.10) for supplemented cows but did no differ at post-partum period (P>0.10). Supplementation did not affect (P>0.10) BCS and calves' BW at calving, and at 45 and 90 days. There was no effect of supplementation on milk yield and composition (P>0.10). There were no differences (P>0.10) with regard forage intake and neutral detergent fiber digestibility. The intake and digestibility of CP and OM increased (P<0.10) with supplementation. Supplementation had no effect (P>0.10) regards serum concentration of metabolites and hormones. Concentration of these variables changed significantly (P<0.10) along the days relative to calving. There was no difference on pregnancy rate and, days from calving to conception among treatments (P>0.10). Under these circumstances, providing 1.5 kg of energetic-protein supplement during the last 60 days of gestation do not improve performance, metabolic and hormonal responses in grazing Nellore cows.

RESUMO

FERREIRA, Matheus Fellipe de Lana, M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Respostas nutricionais, metabólicas e hormonais no periparto de vacas de corte suplementadas no pre-parto**. Orientadora: Luciana Navajas Rennó. Coorientadores: Edenio Detmann e Mário Fonseca Paulino.

O *status* nutricional no parto é o principal fator que afeta o tempo do parto até a concepção. Os parâmetros metabólicos que relacionam o *status* nutricional com processos fisiológicos em animais em pastejo, principalmente zebuínos, não estão totalmente compreendidos. Este estudo avaliou os efeitos da suplementação energético-proteica 60 dias pré-parto sobre o desempenho, as respostas metabólicas e hormonais no periparto de vacas de corte em pastejo. Trinta e oito vacas Nelore multíparas com média de 230 ± 10 dias de gestação foram utilizadas. Foram avaliados dois tratamentos: controle, sem suplementação e suplementação diária (30% de CP) com 1,5 kg durante os 60 dias antes da data do parto esperado. O experimento foi conduzido em delineamento inteiramente casualizado. Foi calculado o ganho médio diário no pré e pós-parto. Os bezerros foram pesados aos 45 e 90 dias. Os escores de condição corporal (ECC) também foram registrados no início do experimento, no parto e 45 dias pós-parto. A cada 30 dias foram coletadas forragem por simulação manual e corte ao nível do solo. Para avaliar o consumo e digestibilidade, um ensaio foi realizado aos 45 dias antes da data estimada de parto. Foi utilizado dióxido de titânio (TiO2) para estimar a excreção fecal e fibra indigestível em detergente neutro (FDNi) para estimar a ingestão de matéria seca de pasto. Assumiu-se que o consumo de suplemento foi igual à quantidade oferecida por animal/dia. No último dia do ensaio, amostras de urina foram coletadas 4 horas antes e após a oferta de suplemento. Nos 30 e 45 dias pós-parto, foi realizado ordenha para estimar a produção de leite. Tendo o parto como dia 0, amostras de sangue foram coletadas antes da suplementação nos dias -30, 0, 15, 30, 45 por punção da veia jugular, utilizando tubos de vácuo para quantificar os teores de glicose, nitrogênio ureico, proteína total, albumina, triglicerídeos, colesterol total, lipoproteína alta densidade (HDL), ácido graxo não-esterificado (AGNE), beta-hidroxibutirato (βHB), insulina, factor de

crescimento semelhante à insulina (IGF-1), triiodotironina total (T3), tiroxina total (T4) e progesterona (P4). Foi calculada a taxa de prenhez e o número de dias do parto até a concepção. O GMD foi maior pré-parto (P<0.10) para os animais suplementados, porém no pós-parto não diferiu (P>0.10). A suplementação não afetou (P>0,10) ECC e PC dos bezerros ao nascimento, e os 45 e 90 dias. Não houve efeito da suplementação sobre a produção e a composição do leite (P> 0,10). Não houve diferenças (P> 0,10) em relação à ingestão de forragem e digestibilidade de fibra em detergente neutro. A ingestão e digestibilidade de proteína bruta e matéria orgânica aumentaram (P <0,10) com suplementação. A suplementação não teve efeito (P> 0,10) em relação à concentração sérica de metabolitos e hormônios. A concentração dessas variáveis mudou significativamente (P <0,10) ao longo dos dias em relação ao parto. Não houve diferença na taxa de prenhez e dias do parto até a concepção entre tratamentos (P>0.10). Nestas condições, fornecer 1,5 kg de suplemento de proteína energética durante os últimos 60 dias de gestação não melhora o desempenho, respostas metabólicas e hormonais nas vacas Nelore em pastejo.

INTRODUCTION

Inadequate nutritional status at calving is the main factor limiting time from calving to conception (Baruselli et al., 2004; Mulliniks et al., 2013), negatively affecting reproductive performance and consequently beef cattle economic success. Accordingly, once beef cows spend most part of pregnancy during dry season period (lower forage yield and quality), energetic-protein supplementation can be adopted in tropical conditions to ensure better performance of grazing cattle (Paulino et al., 2010; Detmann et al., 2014a).

Pre-partum nutrition is more important than postpartum nutrition in the length of postpartum anestrus; and inadequate dietary energy during late gestation impairs reproduction, even when dietary energy is sufficient during lactation. Thereby, the reproductive success of beef cattle is a consequence of pre-partum nutrition. (Hess et al., 2005; Diskin and Kenny, 2016).

It has been recently shown that a period of supplementation 60 days pre-partum had higher efficiency in decreasing the negative energy balance postpartum and reduces the number of days from calving to conception (Silva el al., 2017). However, other experiments do not corroborate, showing that there are still inconsistencies on effect of pre-partum supplementation on cow's performance (Moreno, 2015; Cardenas, 2017).

In general, most part of beef cows' studies with supplementation during gestation did not present extensive analysis of blood hormones and metabolites concentration during peripartum. Those characteristics may help to indicate with accuracy nutritional and physiological changes (Payne & Payne, 1987), being extremely helpful to better understand the effects of supplementation. Therefore, metabolic cues that relate supplementation effects to physiological processes within grazing animals, mainly Zebu, are not fully understood.

The aim of this study was to evaluate the effects of 60-day pre-partum supplementation on performance, metabolic and hormonal responses in peripartum of grazing Nellore beef cows.

HYPOTHESIS

Provide 1.5 kg of energy-protein supplement at 60-day pre-partum improve performance, metabolic and hormonal responses on peripartum of grazing beef cattle.

MATERIAL AND METHODS

Animals, experimental design and treatments

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 31/17).

The experiment was carried out at the facilities of the Beef Cattle Farm of Animal Science Department of University Federal de Viçosa, Viçosa-MG, Brazil. Thirty-eight female pregnant multiparous Nellore cows with 515±11 kg average body weight (BW), body conditions score (BCS) 5,5±0,25 and 230±10 gestation days were used. The animals were randomly divided in eight paddocks averaging seven hectares, evenly covered with *Urochloa decumbens* grass, with free access to water and feeders.

Experimental design was a completely randomized design, with two treatments as following: NS—cows not supplemented during the gestation; SS—cows supplemented during the 60 days pre-partum (from 230 to 290-day period of the gestation). The control, the NS cows, received only a mineral mixture (MM) *ad libitum* during gestation. A total of 90 kg of supplement per animal were provided during the pre-partum period for SS cows (1.5 kg/d) accompanied by a MM offered *ad libitum*. Composition of supplement, MM and pasture are shown in Table 1 and 2.

The supplement was formulated to contain 30% of crude protein (CP) as fed in order to meet around 40% of the CP maintenance requirements, according to BR-CORTE (2016), and provided always at 11h00, to minimize the interference of animal grazing behavior (Adams et al., 1985). After calving, cows remained at

the same paddocks, but only receiving mineral mixture *ad libitum* until 45 days of lactation.

Experimental procedures and sampling

Cows were weighed at the beginning of the experiment (60-days pre-partum), and 7 days before calving to quantify the average daily gain pre-calving (ADGpre). Also, cows were weighted after calving and at the end of the experiment period (45 days) to quantify the average daily gain post-calving (ADGpost). The calves were maintained with dam's during the experiment and weighed immediately after birth, at 45 and 90 days. Body condition scores (BCS) were also recorded on a scale ranging from 1 to 9 as recommended by NRC (1996) by 3 experienced persons at the beginning of the experiment, at calving and 45 days post-partum.

In the breeding season, starting at December 12, cows were synchronized, and fixed time artificial insemination (FTAI) was performed at December 23. The protocol was repeated once more in a way that cows that did not conceive were inseminated again 32 d after the first FTAI. Pregnancy diagnosis was determined via trans-rectal ultrasonography 30 d after FTAI. The number of days from parturition to re-conception was calculated for each cow and pregnancy rate.

Forage sampling

Every 30 days grass samples were collected by hand plucked sampling to evaluate the forage selected by the animals. Also, samples were collected cutting at ground level of five delimited areas of 0.5 x 0.5 m randomly selected in each paddock, to quantify dry matter (DM) and potentially digestible DM (pdDM). Under those circumstances, all samples were weighed, oven-dried (55℃), and then ground to pass through a 1 and 2 mm screen in Wiley mill (model 3, Arthur H.Thomas, Philadelphia, USA)

Intake and digestibility assay

To evaluate intake and digestibility, a trial was performed for nine days at 45 days before the estimated date of parturition (around 245 days of gestation). It was used titanium dioxide (TiO_2) to estimate the fecal excretion of animals,

which was wrapped in paper cartridges in an amount of 20 g per animal/day and inserted with a metal probe via esophagus at 12h00 hours (Titgemeyer et al., 2001). The first five days of trial were used for animal adaptation to TiO_2. Fecal samples were collected immediately after defecation or directly from rectum, in the last 4 days (one sample for day) at the times 18h00, 14h00, 10h00 and 6h00 hours. Feces samples were over-dried (55℃) and ground to pass throug h a 1 and 2 mm screen in Wiley mill (model 3, Arthur H.Thomas, Philadelphia, USA). Then, a quantity of 25 g from each of the 4 days were pooled.

Indigestible neutral detergent fiber (iNDF) was used to estimate the pasture dry matter intake (DMI) (Detmann, et al., 2001). It was assumed that the supplement consumption was equal to the amount offered per animal/day.

At the fifth day of the trial, forage was collected by hand plucked sampling at each paddock separately, and these samples were used to estimate voluntary dry matter intake and digestibility of the forage.

At last day of the trial, spot urine samples (5 mL) were collected 4 hours before and after the supplement offer. Then, a 10 mL compound was prepared with 5 mL of the morning and afternoon urine collected. Urine samples were diluted in 40 mL of H_2SO_4 (0.036 N) and frozen (-20℃).

Milk sampling

At the 30 and 45 days post-calving, were performed milking to estimate the milk production. Aiming to empty the udder, calves were separated from their mothers from 15h00h to 17h45, when they were reunited to the dams and allowed to suckle. At 18h00 calves were again separated from their dams until the next morning. At 6h00 of the next day, cows were milked immediately after an injection of 20 UI of oxytocin (10 UI/mL; Ocitovet®, Brasil) in the mammary vein and the produced milk was weighted. The exact time when each cow was milked was recorded. The calves kept separated from their mother until the next milking at 6h00, to obtain the milk production in 24 hours. It was also separated 30 mL of milk from each cow to evaluate the milk composition and total production was corrected to 4% fat, according to NRC (2001).

Blood sampling

Taking calving day as day 0, blood samples were collected before supplementation on days -30, 0, 15, 30, 45. Blood samples were collected by jugular vein puncture, using vacuum tubes with clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil), to quantity blood nitrogen urea, total protein, albumin, triglycerides, total cholesterol, high density lipoprotein (HDL), non-esterified fatty acid (NEFA), beta-hydroxybutyrate (βHB), insulin, insulin-like growth factor (IGF-1), total triiodothyronine (T3), total thyroxine (T4) and progesterone (P4) contents (only at 30 and 45 days). Tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantity plasma concentration of glucose. After collected, samples were centrifuged at 3600 × g for 20 min, serum and plasma were immediately frozen at -20℃ until analysis.

Laboratory analyses

Samples of forage, feces and supplement were analyzed following procedures described by the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012) for dry matter (DM; index INCT-CA method G-003/1), ash (index INCT-CA method M-001/1), crude protein (CP; index INCT-CA method N-001/1), neutral detergent fiber corrected for ash and protein (apNDF; index INCT-CA method F-002/1) and indigestible neutral detergent fiber (iNDF; Valente et al. 2011) was processed at 2 mm and quantified by in situ incubation procedures with non-woven textile bags (100 g/m²) for 288 hours. Also, fecal samples were evaluated for the contents of titanium (INCT-CA method M-007/1).

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

For blood samples, it was used kits Bioclin® (Belo Horizonte, Brazil) to quantity blood urea nitrogen (BUN) estimated as 46.67% of total serum urea (K056), total protein (K031), albumin (K031), triglycerides (K117), total cholesterol (K083), HDL (K071) and glucose (K082). NEFA and βHB were analyzed using kits Randox® (FA115 and RB1007, Antrim, United Kingdom). All

the analyzes mentioned above were determined by automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). The insulin, Total T3, Total T4 and progesterone contents were analyzed by kits Beckman (33410, 33830, 33800 and, 33550 Beckman Coulter®, Brea, USA). IGF-1 contents were quantified using kits DiaSorin® on automated chemiluminescence analyzer (Liaison®, Italy). Milk was analyzed for protein, fat, lactose, and total solids content, using infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil).

Calculations

Potentially digestible dry matter (pdDM) was estimated using samples collected cutting at ground level, following Paulino et al. (2008) equation:

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

where: 0.98 is the true digestibility coefficient of cell content; NDF is forage content of neutral detergent fiber (%); and iNDF is forage content of indigestible neutral detergent fiber (%).

Fecal excretion (FE) was estimated as a ratio of the TiO2 excreted in feces and marker concentration in feces. Voluntary intake of dry matter of forage (DMF) was estimated using iNDF from the forage as an internal marker, following Detmann et al. (2001) equation:

$$\text{DMF}\left(\frac{\text{g}}{\text{day}}\right) = [(FE * iNDFf) - (SI * iNDFs)]/\, iNDFfo$$

where: FE = fecal excretion (kg/d), iNDFf = indigestible neutral detergent fiber in feces (kg/kg), SI = supplement DM intake (kg/d), iNDFs = indigestible neutral detergent fiber in supplement (kg/kg), and iNDFfo = indigestible neutral detergent fiber in forage (kg/kg).

The daily urine volume was estimated using the relationship between the daily creatinine excretion (CE) and its concentration in the urine. Daily excretion was estimated by equation according to Costa e Silva et al. (2012), where shrunk body weight was estimates as 0.88 x $BW^{1.0175}$ according to Silva et al. (2016).

$$CE\left(\frac{g}{day}\right) = 0.0345 * SBW^{0.9491}$$

Total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in urine, using the equation:

$$Y = \frac{X - 0.301 * BW^{0.75}}{0.8}$$

where, Y= absorbed purines (mmol/d); X = excretion of purine derivatives (mmol/d); 0.301 = endogenous excretion of purine derivate in urine (mmol); $BW^{0.75}$ = metabolic weight; and 0.80 = recovery of absorbed purines as purine derivates in urine (mmol/mmol).

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

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

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

The microbial efficiency was obtained by the ratio between the production of crude microbial protein (PBmic), expressed in grams, and the amount digested organic matter intake (dMO), expressed in kilograms.

Milk production corrected to 4% fat was determined by the equation:

$$FCM = (0.4 * MY) + (0.15 * MY * F)$$

where: MY=milk yield (kg/d) and F= fat yield (%), according NRC (2001).

The serum content of low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according Friedewald et al. (1972); equation: TC= HDL + LDL + VLDL; where TC= total cholesterol e VLDL = triglycerides/5. The globulins were calculated by the difference between total proteins and albumin.

‘

Statistical analyses

The analysis of variance (ANOVA) for the nutritional and performance variables measured during the pre- and post-calving were performed using the following model:

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

where, μ = overall constant; T_i = fixed effect of treatment; $e_{(i)j}$ = random effect of paddock k within the treatment i; $\varepsilon_{(ij)}k$ = random error effect associated to observation l nested in paddock k, which is assumed to be NID (0, σ^2_ε).

The ANOVA for ADG, metabolites and hormones were performed considering repeated measures over time, where the best structure of covariances was chosen using the Akaike's information criterion with correction. The means were compared using Fisher's least significant difference test. Therefore, all statistical evaluations were performed considering 0.10 as the critical level for the occurrence of the type I error. The statistical analyses were performed using the PROC MIXED in SAS 9.4 (Inst. Inc., Cary, NC).

The variables initial body weight and initial BCS were used as co-variables in the model. Before the beginning of the experiment, at the moment of the randomization of the treatments to the experimental units, these variables were tested against the effects of treatments, presenting non-significance. Thus, in the ANOVA, after the end of the experiment, the effects of these variables on animal performance were tested. When not significant, the model was reparametrized without its presence.

Statistical analyses for pregnancy rate were performed using a chi-square test.

RESULTS

Average availability of DM and DMpd during the experiment was 2.74 t/ha and 1.70 t/ha.

There was interaction between supplement and period (pre-partum and postpartum) for ADG (P<0.10). SS cows had higher ADG on pre-partum (P<0.10), but the ADG did no differ between treatments on postpartum period. NS cows ADG did not vary along the periods (P>0.10) (Figure 1).

Supplementation did not affect (P>0.10) calving BW, calving BCS and 45 days post-partum, CBW and calf weight at 45 and 90 days, and days from parturition to conception and pregnancy rate (Table 3). Also, there was no effect of supplementation regarding the milk yield and composition at 30 and 45 days

of lactation (P>0.10) (Table 4). Total DM, organic matter (OM), crude protein (CP) and digestible OM intake were higher (P < 0.10) for supplemented cows, but forage (DMF), apNDF and iNDF intake were not affected (P > 0.10) by treatments (Table 5). However, when expressed in g/kg BW, intakes cited above were not influenced (P > 0.10) by supplement supply.

The supplementation during pre-partum improved (P<0.10) digestibility of OM and CP however it had no effect on apNDF digestibility. Supplementation also did not affect (P>0.10) NMic, but it had effect on the efficiency for synthesis of microbial protein (Emic, g CP/kg dOM), being higher for control animals (Table 6).

There was no interaction (P>0.10) between supplement and days relative to calving towards the blood concentration of glucose, triglycerides, total cholesterol, HDL, LDL and VLDL (Table 7). Besides that, there was no effect of supplementation when these variables were measured (P>0.10). However, the concentration of these variables changed significantly (P<0.10) along the days relative to calving.

For glucose (Figure 2), higher serum concentrations were observed at calving (day 0 - 80,37 mg/dL), decreased at 15 days and then stabilized at baseline (P<0.10). Total cholesterol (Figure 4) and LDL had lower (P<0.10) serum concentrations at calving, then increased from calving to day 30. HDL had higher concentrations at 45. Differently, triglycerides and VLDL serum levels were higher at -30, than calving and post-partum (Figure 3 and 5).

Supplementation did not affect the serum concentration of total proteins, albumin, globulins and creatinine (P> 0.10) during the pre- and post-partum, it had only effect depending on days relative to calving (Table 7).

For total proteins (Figure 6a), serum concentrations were similar at day 30 and 45 (P>0.10), and higher than at day -30, 0 and 15 (P<0.10). For serum albumin (Figure 6b), similar serum concentrations were observed on days -30 and at calving (P> 0.10), however, these were higher than day 15, 30 and 45 (P <0.10). For globulins (Figure 6c), higher serum concentrations were observed at day 45 in relation to the rest of the period, which reflected in the behavior of total proteins. Creatinine decreased throughout the peripartum, with the lowest values on days 30 and 45 postpartum (P<0.10) (Figure 7).

The BUN concentrations presented interaction between supplement and days relative to calving (Table 7), where concentrations were higher for supplemented at day -30 and at calving (P<0.10), and lower at 45 days post-partum (P<0.10) (Figure 8).

Serum concentrations of NEFA were not affected (P>0.10) by the supplementation (Table 7). The NEFA concentration changed depended on the calving day, where concentrations during day -30 were lower than calving day, than stabilized after 30 days (Figure 9). However, there was interaction (P<0.10) between supplementation and days relative to calving for βHB (Table 7), which the lowest concentrations were at -30 for supplemented treatment (Figure 10).

There was no effect of supplementation on insulin and IGF-1 levels, (P>0.10), it only changed along the peripartum days (Table 7), where the peak of both were at calving (Figure 11). On the other hand, both T3 and T4 presented interaction between supplementation and days relative to calving (P<0.10) (Table 7), where concentrations were higher at day -30 for supplemented cows (Figure 12). Supplementation also did not affect the progesterone serum levels (Table 7) which were greater at 45 than 30 days (1.60 and 0.30 ng/mL).

DISCUSSION

Firstly, the supplementation is used to supply the nutrient demand for the animal, which, only pastures are not enough, mainly during the dry season (Paulino et al., 2010; Detmann et al., 2014a). In addition, supplementation can provide changes in ruminal metabolism. More specifically, the use of energetic-protein supplements increases the rate of degradation of the insoluble fibrous compounds, increasing the energy intake extracted from the forage, thus guaranteeing an increase in the total DM intake due to the increase of passage rate (Lazzarini et al., 2009; Sampaio et al., 2009).

Literature data suggest that levels of CP in the diet around 100 g/kg of DM improve fiber degradation (Detmann et al., 2014a), and CP quantity around 145 g/kg DM is able to improve pasture voluntary intake (Detmann et al., 2014b). In this experiment, there was an increase in total DM intake, basically due to addition of the supplement to intake. However, supplementation did not contribute to an increase on pasture intake, and on apNDF digestibility. Basically, in this study, the quantity of CP in the supplement and pasture (around 81 g/kg

MS) was not enough to cause a positive effect on forage voluntary intake and fiber degradation, remaining below to the values suggested by the authors cited above.

On the other hand, supplemented animals had greater CP intake and higher digestibly of OM and CP, which was expected once supplementation provides greater dietary protein intake, besides other high digestible nutrients. As control had lower protein intake and lower OM digestibility, nitrogen utilization efficiency was apparently higher, leading to higher Emic.

Indeed, in the last 60 days of gestation cows present higher requirements (BR-CORTE, 2016), and according to Silva et al. (2017), the supplementation in adequate amounts during this period can have beneficial effect on cow metabolism along with accretion in body reserves. In other words, it seems to metabolically prepare the cow for the postpartum period when supplements are no longer provided. Nevertheless, in this experiment, unlike Silva et al. (2017) the supplementation pre-partum had no effect on reducing the magnitude of BW lost at post-partum, once SS animal presented variation on ADG during experimental period, being negative on post-partum (Figure 1), which also agree with Moreno (2015) and Cardenas (2017).

More, it was not observed difference in the length of postpartum anestrus, once there was no effect of supplementation in progesterone levels (Table 7), which indicates that possibly supplementation was not able to contribute to greater reproductive efficiency. As expected, results cited above reflected consequently on post-partum performance, presenting also no difference on pregnancy rate and days from calving to conception. Probably, those results can be explained by the fact that most of the animals started the experiment with appropriate BCS for reproduction (5 to 6.0 - on a scale of 1 to 9) (Lowman,1976; Randel, 1990).

According to several works, BCS is a determinant factor for cows to return to early estrus, improving conception rates (Bishop et al., 1994; Hess el al., 2005). Furthermore, for cows with adequate BCS, there is evidence that body reserves can be used during late gestation without compromising subsequent reproductive function (Diskin & Kenny, 2016), thus questioning the need for supplementation of cows with adequate BCS at the end of gestation. In other words, if the animal

is already in good condition to reproduction (i.e. BCS, 5 to 6), the supplementation, regardless its period, will not lead to great performance.

In addition, although the pasture had lower quality, it had sufficient availability for animals, making possible a selective grazing, which led NS animals to maintain ADG throughout the experimental period (Figure 1). Therefore, it resulted also on neither difference on calves' body weight, and milk production. According to Marques et al. (2016), females that has adequate BCS throughout pregnancy do not influence the body weight of the progeny at birth. Similar results are also related by Marquez et al. (2017), and Trece (2017) who did not find effect of supplementation on calves' birth weight and calves average daily gain.

In fact, those affirmations also can be supported by the results of hormones and metabolites, which, for the most part, it only varied according to peripartum days.

It was observed a decrease of plasma glucose concentration, regardless supplementation, at the end of gestation, which can be explained by the higher fetal demand at this period (BR-CORTE, 2016). At calving, cows are under stressful conditions, thus epinefrine act stimulating glycogen catabolism (Kolnes, et al. 2014) in order to minimize the stress during calving, while glucocorticoids act promoting gluconeogenesis in liver, whereas in muscle and adipose tissue they decrease glucose uptake and utilization (Foster, 1988). Therefore, the glucose concentrations reached higher levels at this period. Decreased glucose concentrations after calving were probably caused by reduced DM intake, and also to the higher energy demand for milk production, using glucose to produce lactose (Larson, 1985). At days 30 and 45, serum levels of glucose were restored and maintained on basal levels.

There was a progressive increase in cholesterol levels throughout the post-partum days regardless the supplementation period, also related by Ruas et al. (2000) and Godoy et al. (2004) on postpartum blood cholesterol in lactating beef cows. Similarly, occurs with HDL concentrations, which also increases after calving. In ruminants, lactogenesis increases plasma HDL concentration, possibly due to an increase in HDL synthesis or an increase in catabolism of VLDL by mammary tissue (Puppione, 1978), which explains the decreased triglycerides and VLDL concentrations at post-partum, suggesting its utilization

as energy demand for lactation, once they are an important source of fatty acids for the milk fat synthesis (Aeberhard et al., 2001).

Moreover, the increase in cholesterol during postpartum might be related to the need for precursors for the synthesis of steroidal hormones (Holtenius et al., 2003). During the reestablishment of reproductive activity, avascularized granulosa cells are restricted to cholesterol uptake from HDL (Shalgi et al., 1973), therefore, there's higher need of HDL, over VLDL and LDL.

Literature data shows that pregnant cows feed restricted during the last gestation period are susceptible to weight loss, BCS loss and high serum concentrations of NEFA and βHB, leading to long periods of negative energy balance, both in dairy (Bell, 1995; Barber et al., 1997; Bauman, 2000) and beef cows (Mulliniks et al., 2013).

In this study, NEFA concentrations were not affected by supplementation and its levels at calving indicates increased adipose tissue rate of lipolysis (Oetzel, 2004; Astessiano et al., 2013; Lopes et al., 2016). NEFA post-partum concentrations were lower compared to parturition, remaining at basal concentrations during the experimental period, suggesting recovery of the nutritional status of the animals. Similarly, same occurs with βHB levels at post-partum, however, it was different between treatments at pre-partum, showing higher concentration for control animals.

Despite the difference between treatments in βHB concentrations at -30 days, those levels do not indicate intense body reserves mobilization for control animals, even ADG presenting difference among treatments at pre-partum. It's important to emphasize that, the most part of studies of energy deficit in ruminants are with dairy cows, and despite the lack of information regarding beef cows' serum βHB levels, it is possible to understand that those levels found in this experiment, regardless supplementation, do not suggest severe energy deficit, but suggest that cows were not in similar nutrient balances at pre-partum.

Nevertheless, βHB levels do not influenced on days from calving to conception, unlike Mulliniks et al. (2013) that found that reduced concentration of βHB was associated with an earlier conception date in beef cows. The contrasting results in experiments may be explained by the fact that βHB values that impaired reproduction found by Mulliniks et al. (2013) at -30 days were higher (0.71 mmol/L) than values found in this experiment (0.48 mmol/L).

The albumin concentration presented a significant decreased after calving, which possibly related to the demand for amino acids for milk production (Contreras, 2000). Although at 45-days it had the lowest value found, it stills remained within the references values (3.03-3.55 g/dL), according Kaneko et al. (1997). At 30 days pre-calving and at calving, globulin was decreased in relation to the rest of the period, justified by the transfer of immunity to colostrum production (Weaver et al., 2000), which reflected in the behavior of total proteins concentrations.

Unlike the others protein status indicators, BUN levels were higher for supplemented animals during only the pre-partum period, basically due to the effect of supplementation, which raised ammonia in the rumen. As urea is considered a short-time protein indicator, it was expected higher BUN levels during supplement period. Urea is synthesized in the liver in amounts proportional to the concentration of ammonia produced in the rumen and its concentration is directly related to dietary protein levels (Wittwer et al., 1993; Owens & Bergen, 2011).

The blood concentration of creatinine is an index of muscle mass, in way that creatinine excretion is proportional to lean body mass, and thereby proportional to the animal's BW (Lofgreen and Garret, 1954). As expected, creatinine concentrations decreased linearly throughout the peripartum, due weight loss (VandeHaar et al., 1999), with the lowest values on days 30 and 45 post-partum. Yet, it stills into the references values (1-2 mg/dL, Kaneko et al., 2008).

The nutritional status also regulates the production of IGF-I and insulin, which are sensitive to plane of nutrition (Clemmons et al., 1985). Feed restricted animals have lower serum glucose, and by consequence, lower insulin; which causes reduction in somatotropin (GH) receptors in the liver, the main mediator of IGF-I production. Thus, animals in the catabolic state have lower concentrations of plasma IGF-I (McGuire et al., 1992; Thissen et al.,1994; Yambayamba et al., 1996). Therefore, IGF-I and insulin are physiologically linked and both increase with enhanced BCS. However, the regulation of each hormone individually may vary according to metabolic status, and/or the direction of the changes in body weight.

During the experiment, insulin and IGF-1 levels behavior were similar and not influenced by supplementation. In similar conditions, Silva et al. (2017) found no difference on insulin levels of cows supplemented during pre-partum. In addition, Trece (2017) also did not find effect of pre-partum supplementation on both insulin and IGF-1 concentrations. The higher values for both hormones were during calving day, possibly due to the increase of blood glucose and its decrease during early lactation is part of the homeorhetic changes to support galactopiesis (Bauman, 2000). Thereafter, IGF-1 concentrations were restored after 30 days post-partum, and insulin at 45 days, thereby stimulating steroidogenesis (Wettemann, 2000) and leading to higher progesterone levels at 45 days.

Ruminants during food restriction adapt lowering the maintenance requirements by decreasing basal metabolism rate (Chilliard et al., 1998), due decreased circulating levels of thyroid hormones. In this experiment, there was a reduction of both total T3 and T4 on pre-partum for NS animals, possibly explained by a decreased metabolic rate, compared to SS.

Several works have notice also that cows in post-partum negative energy balance respond to reduce the concentrations of total T3 and T4, due to the energy deficiency state as well as to the large demand for these hormones by the mammary gland (Ronge et al., 1988; McGuire et al., 1991). Differently, from parturition to 45 days, T3 and T4 total levels had distinct behavior. Coggins and Field (1977) demonstrated that, compared to T3, serum concentrations of T4 were a more sensitive indicator of energy balance in lactating beef cows. This support observation in this experiment of lowered levels of T4 in 15 days post-partum, unlike T3 that did not vary much during post-partum.

Overall, there were no carry over effects of 60-day pre-partum supplementation on post-partum physiological response.

CONCLUSIONS

In livestock production systems, where grazing Nellore cows present satisfactory BCS at the end of gestation, provide 1.5 kg energetic-protein supplement during the last 60 d of gestation does not improve performance during peripartum. Moreover, supplementation during pre-partum does not

influence in hormones and metabolites levels, which mostly vary along the peripartum days.

REFERENCES

AEBERHARD K, BRUCKMAIER R M, BLUM J W. Metabolic, enzymatic and endocrine status in high yielding dairy cows – Part 2. **Journal of Veterinary Medicine.** A, Physiology, Pathology, Clinical Medicine, v.48, p.111–127. 2001.

ADAMS, D. C. Effect of time of supplementation on performance, forage intake and grazing behavior of yearling beef steers grazing Russian wild ryegrass in the fall. **Jounal of Animal Science**. v.61. n.5. p.1037-1042.1985.

ASTESSIANO, A. L.; PÉREZ-CLARIGET, R.; ESPASANDÍ, A.C. et al. Metabolic, productive and reproductive responses to postpartum short-term supplementation in primiparous beef cows. v.42, n.4, p.246-253, 2013.

BARBER M.C. et al. Lipid metabolism in the lactating mammary gland. **Biochimica et Biophysica Acta.** 1347. p.101–126, 1997.

BARBOSA, A.M.; VALADARES R. F.D.; VALADARES FILHO S.C. et al. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle. **Journal of Animal Science**, v.89, p.510-519, 2011.

BARUSELLI P. S., REIS E. L., MARQUES M. O. et al. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. **Animal Reproduction Science**, v.120, p.479-48, 2004.

BAUMAN D.E., Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. In **Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction. Proceedings of the 9th**

International Symposium on Ruminant Physiology, p. 311–328. Ed. PB Cronje. New York: CABI Publishing. 2000.

BELL A.W., Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. **Journal of Animal Science**, v.73 2804–2819,1995.

BISHOP, D. K., R. P. Wettemann, and L. J. Spicer. Body energy reserves influence the onset of luteal activity after early weaning of beef cows. **Journal of animal science** v.72 p.2703-2708, 1994

CARDENAS J.E.G. Nutritional and metabolic evaluation of nellore cows supplemented or not during the peripartum. 2017. 44f. Tese (Doutorado em Zootecnia) - Universidade Federal de Viçosa, Minas Gerais. 2017

CHAPMAN, M. J. Animal lipoproteins: chemistry, structure, and comparative aspects. **J. Lipid Res**. v.21 p-789.1980

CLEMMONS, D. R; DEHOFF, M.; MCCUSKER, R.; et. al. The role of insulin-like growth factor in the regulation of growth. **Journal of Animal Science**, Suppl-2, p. 165-170, 1987.

CHEN, X.B.; GOMES, M.J. Estimation of microbial protein supply to sheep and cattle basid on urinary excretion of purine derivatives-an overview of the technical details. **Ocasional publication. Buchsburnd Aberdeen**. Ed. Rowett Research Institute. 21p. 1992.

CHILLIARD Y.; BOCQUIER, F.; DOREALL, M. et al. Digestive and metabolic adaptation of ruminants to undernutrition and consequences on reproduction. **Reproductive Nutrition Development**, No.38, v.2, p131-152. 1998

COGGLNS, C. R. and A. C. HELD. Changes in plasma concentrations of glucose, free fatty acids, ketone bodies, thyroxine and insulin of lactating beef cows in relation to time of feeding and energy status. In: D. Lister (Ed.) Blood Profiles in Animal Production. **British Society of Animal Production**. No. 1, p 41-47,.1977.

COLLIER, R.J. 1985. Nutritional, metabolic, and environmental aspects of lactation. In: B.L. Larson (Ed.) Lactation. Iowa State University Press, Ames. p80-128

CONTRERAS, P. Indicadores do metabolismo proteico utilizados nos perfis metabólicos de rebanhos. GONZÁLEZ, F.H.D.; BARCELLOS, J.O; OSPINA, H.; RIBEIRO, L.A.O. (Eds). **Perfil Metabólico em ruminantes: seu uso em nutrição e doenças nutricionais**. Porto Alegre, Brasil, Gráfica da Universidade Federal do Rio Grande do Sul, 2000.

DETMANN, E., SOUZA, M.A., VALADARES FILHO, S.C., et al. (Ed.) (2012). Métodos para análise de alimentos. 1.ed**. Instituto Nacional de Ciência e Tecnologia de Ciência Animal**. Visconde do Rio Branco: Suprema, 214p.

DETMANN, E., PAULINO MF, ZERVOUDAKIS JT, et al. Chromium and internal markers in intake determination by crossbred steers, supplemented at pasture. **Revista Brasileira de Zootecnia** v.30, p.1600-1609, 2001.

DETMANN, E., S.C. VALADARES FILHO, PAULINO, M.F., HUHTANEN, P. Nutritional aspects applied to grazing cattle in tropics: a review based on Brazilian results. **Semina: Ciências Agrárias**, v.35 p.2829-2854, 2014a

DETMANN, E., VALENTE, E.E.L., BATISTA, E.D., P. HUHTANEN. An evaluation of performance and efficiency of nitrogen utilization in cattle feed tropical grass pastures with supplementation. **Livestock Science**, v.162, p.141-153, 2014b.

DETMANN, E., PAULINO, M.F., VALADARES FILHO, S.C., 2010. Otimização do uso de recursos forrageiros basais. In: **Anais do 7º Simpósio de Produção de Gado de Corte,** Viçosa. p.191-240.

DISKIN, M.G., KENNY, D.A. Managing the reproductive performance of beef cows, **Theriogenology**, v.86, p.379-387, 2016.

FOSTER, L.A., Clinical ketosis. **Veterinary Clinics of North America-Food Animal Practice** 4, p.253–267, 1988.

FRIEDEWALD, W.T., LEVY, R.I., FREDRICKSON, D.S. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. **Clinical Chemistry**. v.18, p.499–502, 1972.

GODOY, M.M.; ALVES, J.B.; MONTEIRO, A.L.G. et al. Reproductive and Metabolic Parameters of Guzerá Cows Supplemented in Pre and Postpartum. **Revista Brasileira de Zootecnia**, n.1, v.33, p.103-111, 2004.

HESS, B.W., LAKE, S.L., SCHOLLJEGERDES, E.J. Nutritional controls of beef cow reproduction, **Journal of Animal Science**, v.83, p.90-106, 2005.

HOLTENIUS, K, AGENAS, S, DELAVAUD, C, CHILLIARD, Y. Effect of feeding intensity during the dray period. 2. metabolic and hormonal responses. **Journal of Dairy science**, v. 86, p. 883-891, 2003.

LARSON, B.L. Biosynthesis and cellular secretion of milk. In: B. L. Larson (Ed.) **Lactation. Iowa State University Press**, Ames. p.129-163, 1985.

LAZZARINI, I.; DETMANN, E.; SAMPAIO, C.B. et al. Consumo, digestibilidade e dinâmicas de trânsito e degradação da fibra em detergente neutro em bovinos alimentados com forragem tropical de baixa qualidade e compostos nitrogenados. **Arquivo Brasileiro de Medicina Veterinária**, v.61, p.635-647, 2009.

LOFGREEN, G.P., GARRET, W.N., Creatinine excretion and specific gravity as related to the composition of the 9, 10, 11th rib cut of Hereford steers. **J. Anim. Sci**. v.13, p.496–500, 1954.

LOPES, S.A.; PAULINO, M.F.; DETMANN, E.; RENNÓ, L.N.; VALENTE, E.E.L.; CABRAL, C. H. A.; CARVALHO, V.V.; LIMA, J.A.C.; MANSO, M.R.; BONFÁ, H. C. Evaluation of grazing beef cows receiving supplements with different protein contents. **Semina: Ciências Agrárias**, v.37, p.3361-3372, 2016.

LOWMAN B.G., et al. Condition scoring of cattle. Edinburgh, UK: East of Scotland **College of Agriculture**; No 6. 1976.

LUCY, M.C. Mechanisms linking nutrition and reproduction in postpartum cows. **Reproduction**, v.61, p.237-254, 2003.

KANEKO, J.J.; HARVEY, J.W.; BRUSS, M.L. Clinical biochemistry of domestic animals. San Diego, **Academy Press.** 1997.

KOLNES, A.J.; BIRK., J.B.; EILERTESEN E. et al. Epinephrine-stimulated glycogen breakdown activates glycogen synthase and increases insulin-stimulated glucose uptake in epitrochlearis muscles. **A'm J Physiol Endocrinol Metab.** v.308 p.231-240. 2015.

McGUIRE, M. A., J. L. VICINI, D. E. BAUMAN, AND J. J. VEENHUIZEN. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. **Journal of Animal Science.** v.70, p.2901– 2910, 1992.

McGUIRE, M. A., W. S. COHICK, D. R. CLEMMONS, et al. 1991. Effects of protein and energy restriction on concentrations of IGF and IGF binding protein-2 (IGFBP-2) in lactating cows treated with somatotropin (IbST). (Abstr.) **In 2nd Int. Symp**. Insulin-like Growth Factors/ Somatomedins. p 148.

MARQUES, R. S., COOKE R. F., RODRIGUES M C, et al. Impacts of cow body condition score during gestation on weaning performance of the offspring. **Livestock Science**, v.191, p.174–178, 2016.

MARQUEZ, D. C., PAULINO, M. F., RENNÓ, L. N., et al. Supplementation of grazing beef cows during gestation as a strategy to improve skeletal muscle development of the offspring. **Animal**, p.1-9, 2017

MORENO, D.S. Productive and metabolic response of beef heifers in tropical conditions with strategic prepartum and postpartum supplementation. **Dissertação (Mestrado em Zootecnia)** - Universidade Federal de Viçosa, Minas Gerais. 2015.

MULLINIKS J. T., KEMP M. E., ENDECOTT R. L. et al. Does β-hydroxybutyrate concentration influence conception date in young postpartum range beef cows. **Journal of animal science**. v.91 p.2902-2909. 2013

NATIONAL RESEARCH COUNCIL - NRC. Nutrient Requeriments of Beef Cattle. 7.ed. **National Academy Press**, Washington, D.C., 2000. 244p.

NATIONAL RESEARCH COUNCIL - NRC. Nutrient requeriments of dairy cattle. 7.ed. **National Academy of Science,** Washington,D. 2001. 381p.

PAULINO, M.F. et al. Bovinocultura programada. In: VII SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 7, 2010, Viçosa**. Anais...**Viçosa: DZO-UFV, 2010. p.267-297

PAULINO, M.F. et al. Nutrição de bovinos em pastejo. In: SIMPÓSIO SOBRE MANEJO ESTRATÉGICO DA PASTAGEM, 4, 2008, Viçosa. **Anais...** Viçosa: DZO-UFV, 2008. p.131-169.

OETZEL, G.R. Monitoring and testing dairy herds for metabolic diseases. Veterinary Clinics of North America. **Food Animal Practice**, Philadelphia. v20, p.651 -674, 2004.

OLSON, B.R.; CARTLEDGE, T.; SEBRING, N. Short-term fasting affects luteinizing hormone secretory dynamics but not reproductive function in normal-weight sedentary women. **Journal of clinical endocrinology and metabolism**, v. 80, p. 1187-1193, 1995.

OWENS F. N. and BERGEN W. G. Nitrogen metabolism in ruminant animals, historical perspective, current understanding and future implications. **Journal of animal science**. v. 57, Suppl. 2, 1983.

RANDEL D.R. Nutrition and postpartum rebreeding in cattle. **Journal of animal Science**. v.68, p 853-862. 1990.

RONGE, H., J. BLUM, C. CLEMENT, et al. 1988. Somatomedin C in dairy cows related to energy and protein supply and to milk production. **Animal Production**. v47, p165, 1988.

RUAS, J.R.M.; TORRES, C.A.A.; VALADARES FILHO, S.B. et al. Efeito da suplementação proteica a pasto sobre consumo de forragens, ganho de

peso e condição corporal, em vacas Nelore. **Revista Brasileira de Zootecnia,** , n.3, v.29, p.930- 934, 2000.

SAMPAIO, C.B.; DETMANN, E.; LAZZARINI, I. et al. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Revista Brasileira Zootecnia**, v.38, p.560-569, 2009.

SILVA, A. G, PAULINO, M.F., DETMANN, E., et al. Energetic-protein supplementation in the last 60 days of gestation improves performance of beef cows grazing tropical pastures. **Journal of Animal Science and Biotechnology** v.8, p.78. 2017

SILVA, L.F.C; VALADARES FILHO, S.C; CHIZZOTTI, M.L; et al. Creatinine excretion and relationship with body weight of Nellore cattle. **Revista Brasileira de Zootecnia**, v. 41, p. 807-810, 2012.

SHALGI, R.; KRAICER, P.; RIMON, A.; PINTO, M. and SOFERMAN, N.1973. Protein of human follicular fluid: the blood follicle barrier. **Fertil. Steril**. v.24, p.429.

PAYNE, J.M.; PAYNE, S. **The metabolic profile test.** Oxford, oxford University Press. 1987.

PUPPIONE, D. L. Implications of unique features of blood lipid transport in the lactating cow. **Journal of Dairy Sciece**. v.61, p-651, 1978.

TITGEMEYER, E.C., ARMENDARIZ, C.K., BINDEL, D.J. et al. Evaluation of titanium dioxide as a digestibility marker for cattle, **Journal of Animal Science**, v. 79, p.1059-1063, 2001.

THISSEN, J.P., KETELSLEGERS J. M., and UNDERWOOD, L. E. Nutritional regulation of the insulin-like growth factors. **Endocr. Rev.** v.15, p.80–101, 1994.

TRECE, A S. Avaliação nutricional e metabólica em vacas de corte suplementadas no pré e/ou pós-parto. **Dissertação (Mestrado em Zootecnia)** - Universidade Federal de Viçosa, Minas Gerais. 2017

VALADARES FILHO SC, COSTA E-SILVA LF, GIONBELLI MP, ROTTA PP, MARCONDES MI, CHIZZOTTI ML. **BR-CORTE 3.0 - Nutrient requirements of zebu and crossbred cattle**. 3rd Ed. Viçosa: Universidade Federal de Viçosa; 2016

VALENTE, T.N.P., DETMANN, E., QUEIROZ, A.C. et al. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. **Rev. Bras. Zootec**. v.40, p2565–2573, 2011.

VANDEHAAR, M.J.; YOUSIF, G.; SHARMA, B.K.; HERDT, T.H.; EMERY R.S.; ALLEN, M.S.; and LIESMAN, J.S.1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. **Journal of Dairy Sciece**. 82:1282–1295

YAMBAYAMBA, E. S.; PRICE, M. A.; FOXCROFT, G. R. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers**, Journal of Animal Science**, p. 74. 57-69, 1996.

WEAVER, D. M., TYLER, J. W., VANMETRE, D. C. et al. Passive transfer of colostral immunoglobulins in calves. **Journal of Veterinary Internal Medicine**. v.14, p.569 – 577, 2000

WETTEMANN RP, Bossis I. Energy intake regulates ovarian function in beef cattle. **Journal of Animal Science**. v. 77(Suppl 1) p1–10. 2000.

WITTWER, F.; REYES, J.M.; OPITZ, H. et al. Determinación de urea en muestras de leche de rebaños bovinos para el diagnostico de desbalance nutricional. **Archivo Medico Veterinario**. v. 25, p. 165-172, 1993.

Table 1. Ingredients and composition of supplement provided to cows at 60-days pre-partum

Item[1]	Supplement
Ingredients (%; as-fed basis)	
Corn meal	41.2
Soybean meal	36.0
Wheat meal	20.0
Urea:ammonium sulfate (9:1)	2.80
Chemical composition (g/kg of DM)	
OM	965
CP	320
apNDF	143

[1] OM – organic matter; CP – crude protein; apNDF – neutral detergent fiber corrected for ash and protein residue.
Mineral mix - CaHPO4= 50.00%; NaCl= 47.775%; ZnSO4= 1.4%; Cu2SO4= 0.70 %; CoSO4= 0.05%; KIO3= 0.05% and MnSO4= 0.025%.

Table 2. *Uruchloa decumbes* chemical composition

Item	Months			
	August[4]	September	October	November
DM[1]	651.3	762.3	505.8	236.7
OM[2]	931.8	937.6	934.1	910.4
CP[2]	48.8	52.3	58.3	82.1
apNDF[2]	749.3	770.1	731.2	592.5
iNDF[2]	338.7	347.6	362.1	177.8
NDIN[3]	217.1	190.4	259.2	425.6

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre corrected for ash and protein (apNDF), indigestible neutral detergent fiber (iNDF), insoluble neutral detergent nitrogen (NDIN).
[1]/ g/kg of natural matter
[2]/ g/kg DM
[3]/ g/kg total nitrogen
[4]/ trial

Table 3. Least square means and P-values for effect of energy-protein supplementation on cows and calves' performance

Item[1]	Treatment		SEM	P-value
	No supplementation	Supplementation		
Calving BW (kg)	515.1	536.9	13.86	0.315
Calving BCS	5.19	5.53	0.222	0.233
BCS 45	5.38	5.11	0.273	0.492
CBW (kg)	30.8	32.1	1.14	0.442
CBW45 (kg)	70.6	73.0	1.78	0.380
CBW90 (kg)	93,2	101.1	6.30	0.409
N° days to conception	80	77	11.30	0.874
Pregnancy rate (%)	73	68	-	0.720

[1]BW- body weight; BCS – Body Condition Score; ADG - Average Daily Gain; CBW - Calf Birth Weight; CBW45- Calf Body Weight at 45 days; CBW90- Calf Body Weight at 90 days

Table 4. Least square means and P-values for effect of energy-protein supplementation on milk production and composition

Item[1]	Treatment		SEM	P-value
	NS	SS		
Milk 30	7.69	7.84	0.432	0.817
FCM 30[a]	8.68	8.43	0.521	0.742
Fat (%)	5.00	4.90	0.205	0.755
Protein (%)	2.99	3.02	0.054	0.697
Lactose (%)	4.60	4.65	0.066	0.557
Total solids (%)	13.40	13.31	0.285	0.825
Milk 45	7.74	8.15	0.424	0.525
FCM 45[a]	9.17	9.41	0.449	0.717
Fat (%)	5.40	5.17	0.244	0.514
Protein (%)	3.05	3.09	0.057	0.598
Lactose (%)	4.63	4.65	0.047	0.673
Total solids (%)	13.89	14.07	0.221	0.556

[a]FMC=4% fat-corrected milk yield (30 and 45 days)

Table 5. Least square means and P-values for effect of energey-protein supplementation on cow's intake during pre-calving

Itens	Treatments		SEM	*P*-value
	No supplementation	Supplementation		
	kg/d			
DM	7.82	8.88	0.317	0.056
DMF	7.82	7.54	0.317	0.555
OM	7.29	8.29	0.291	0.052
CP	0.38	0.80	0.015	<0.001
apNDF	5.96	5.86	0.237	0.775
iNDF	2.66	2.57	0.105	0.569
dOM	2.93	3.91	0.227	0.018
dNDF	3.02	2.94	0.171	0.751
	g/kg BW			
DM	15.33	17.24	0.742	0.118
DMF	15.33	14.63	0.718	0.518
OM	14.31	16.09	0.684	0.114
apNDF	11.68	11.39	0.539	0.701
iNDF	5.22	4.99	0.236	0.523

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

Table 6. Least square means and P-values for effect of energey-protein supplementation on apparent digestibility and synthesis of nitrogen compounds during pre-calving

Itens	Treatments			*P*-value
	No supplementation	Supplementation	SEM	Sup
OM	39.86	47.32	1.522	0.013
CP	2.77	50.65	1.623	<0.001
apNDF	50.67	50.21	0.014	0.832
Nmic	89.24	92.69	66.535	0.370
Emic	190.06	148.16	12.666	0.036

Organic matter (OM, %), crude protein (CP, %), neutral detergent fiber corrected for ash and protein (apNDF, %), digested organic matter (dOM, g/kg DM), ruminal synthesis of microbial nitrogen (NMic, g/d), efficiency for synthesis of microbial protein (Emic, g microbial CP synthesis kg dOM intake).

Table 7. Least square means and P-values for effect of supplementation on serum metabolites and hormones during pre and post-calving

Itens	Treatments		SEM	*P*-value		
	No supplementation	Supplementation		Sup[2]	Day[3]	Sup x Day
Glucose, mg/dL	62.97	63.70	1.895	0.793	<0.001	0.124
Triglycerides, mg/dL	26.74	27.07	1.085	0.835	<0.001	0.316
Total cholesterol, mg/dL	132.04	142.47	5.385	0.219	<0.001	0.458
VLDL mg/ dL,	5.34	5.41	5.385	0.219	<0.001	0.458
LDL mg/ dL,	56.21	66.15	5.054	0.213	<0.001	0.558
HDL mg/ dL,	69.45	70.58	3.339	0.819	<0.001	0.309
Creatinine, mg/dL	1.40	1.39	0.054	0.754	<0.001	0.289
BUN, mg/dL	14.46	15.18	0.836	0.562	<0.001	<0.001
Total Proteins, g/dL	7.39	7.43	0.124	0.821	<0.001	0.436
Albumin, g/dL	3.26	3.24	0.042	0.723	<0.001	0.735
Globulins, g/dL	4.15	4.19	0.149	0.867	<0.001	0.453
NEFA, mmol/L[1]	0.33	0.27	0.042	0.377	<0.001	0.206
βHB, mmol/L[1]	0.47	0.45	0.021	0.676	0.073	0.026
IGF-1, ng/dL	184.64	196.54	16.670	0.629	<0.002	0.360
Insulin, µIU/mL	2.99	2.83	0.324	0.737	0.0019	0.806
T3, ng/mL	0.637	0.823	0.1305	0.350	0.006	0.025
T4, µg/dL	4.66	5.81	0.689	0.282	<.001	<.001
Progesterone, ng/mL,	0.87	1.02	0.2461	0.687	0.0008	0.674

[1]/Non-esterified fatty acids (NEFA); β-hydroxybutyrate (βHB)
[2]/ Supplementation (Sup)
[3]/ Day relative to calving (Day)

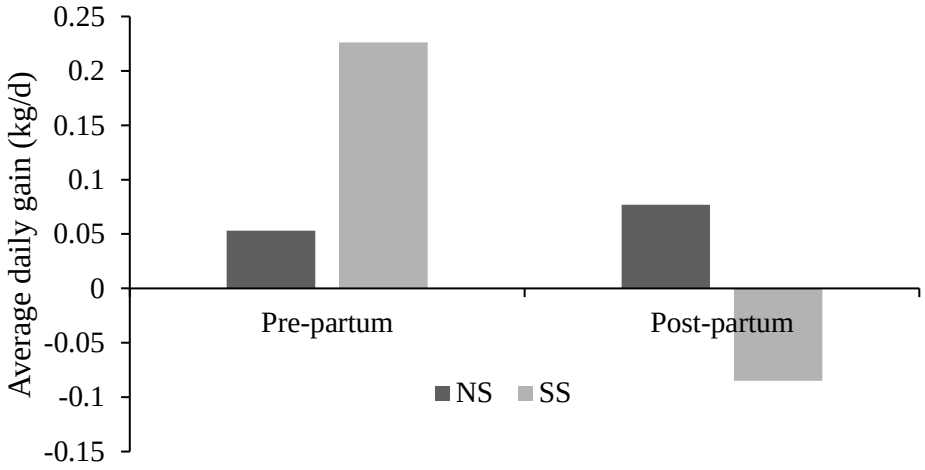


Figure 1. Average daily gain on pre- and post-partum period.

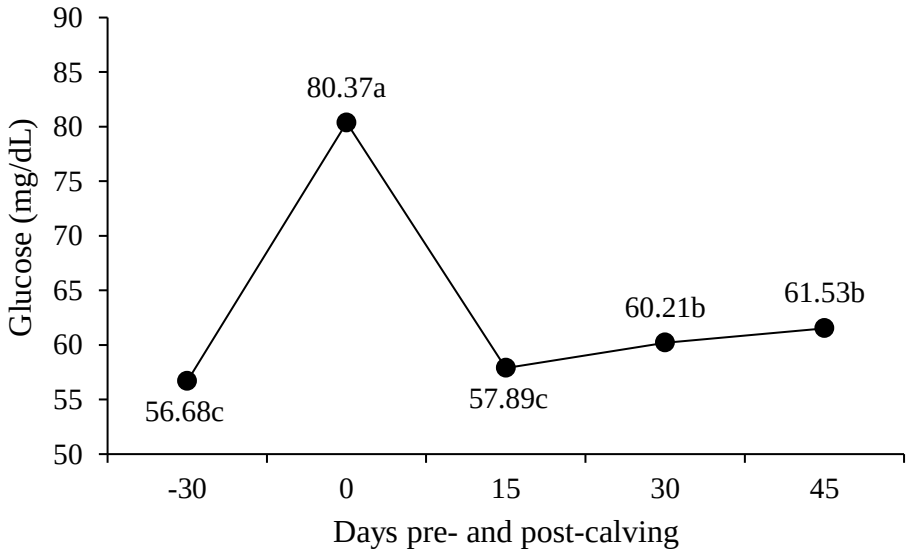


Figure 2. Glucose plasma concentrations during pre- and post-calving. Different letters declare significantly different serum concentration of glucose (P<0.10).

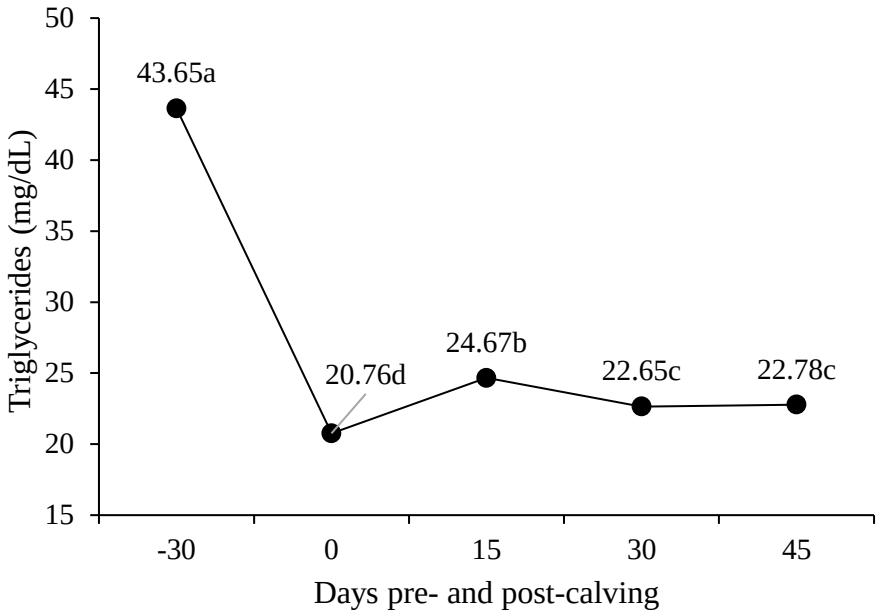


Figure 3. Triglycerides serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentrations (P<0.10).

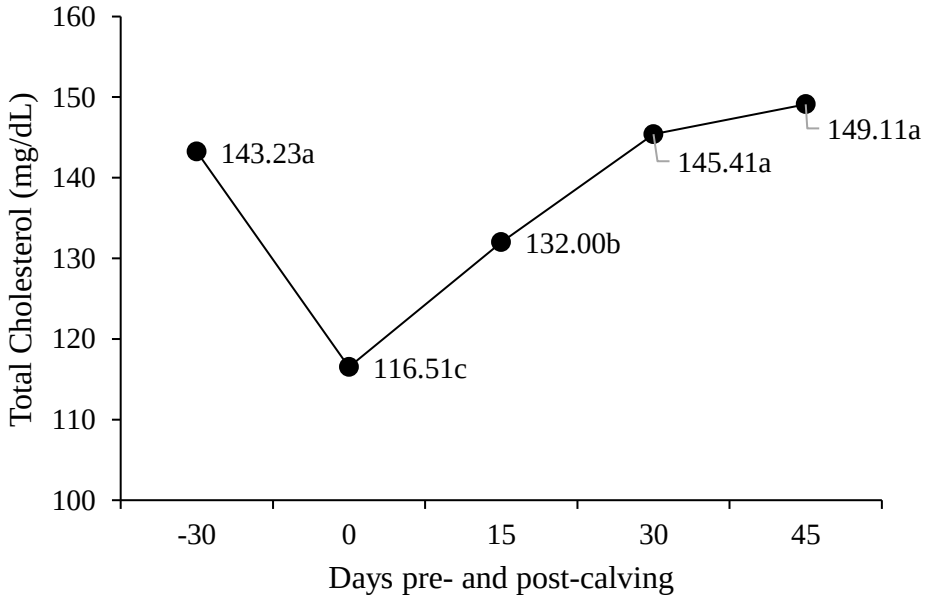
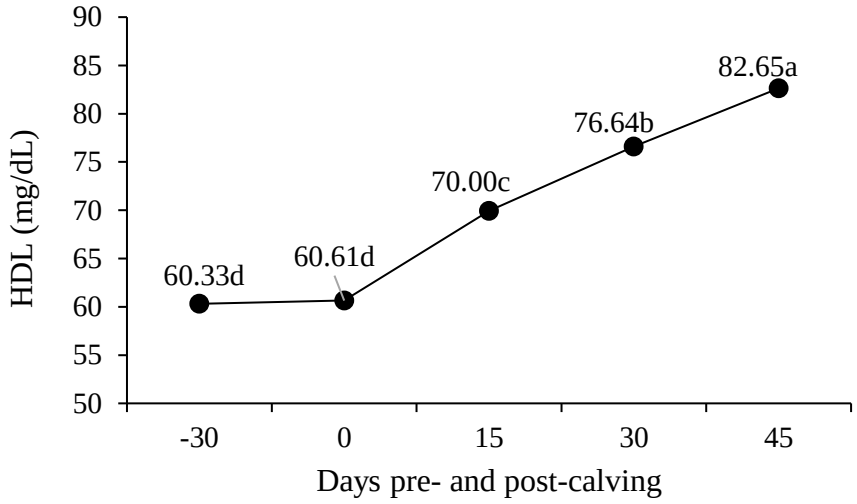
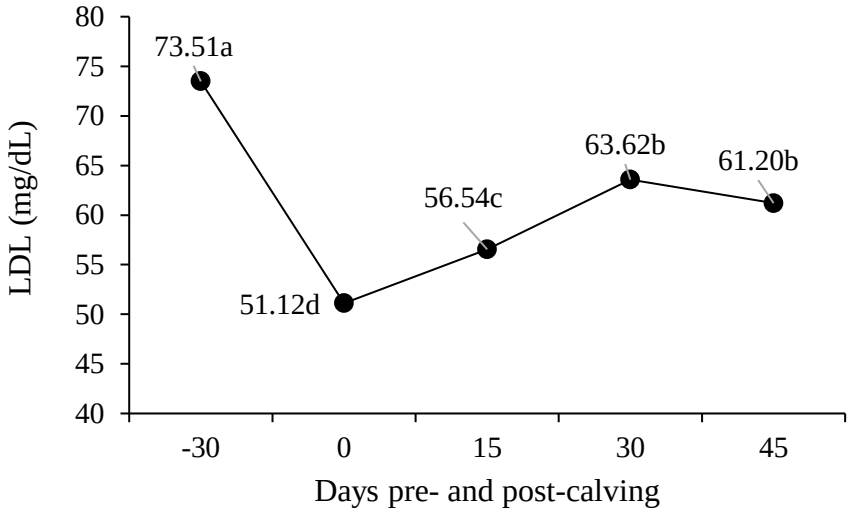


Figure 4. Total cholesterol serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentrations (P<0.10).

(a)



(b)



(c)

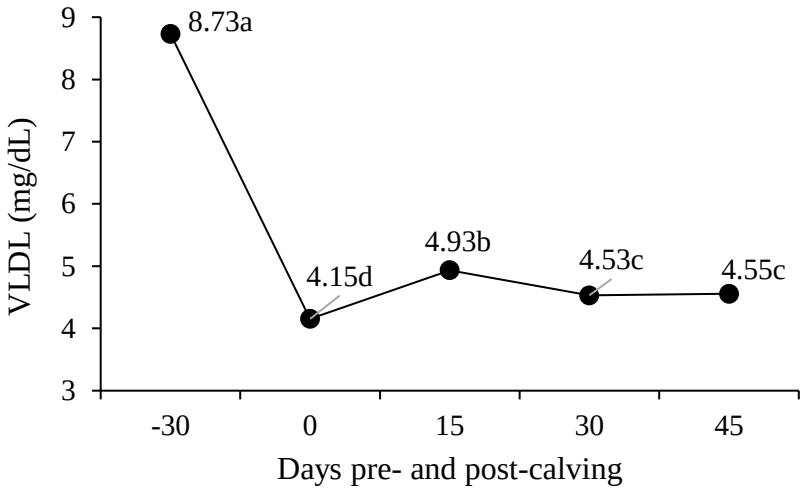
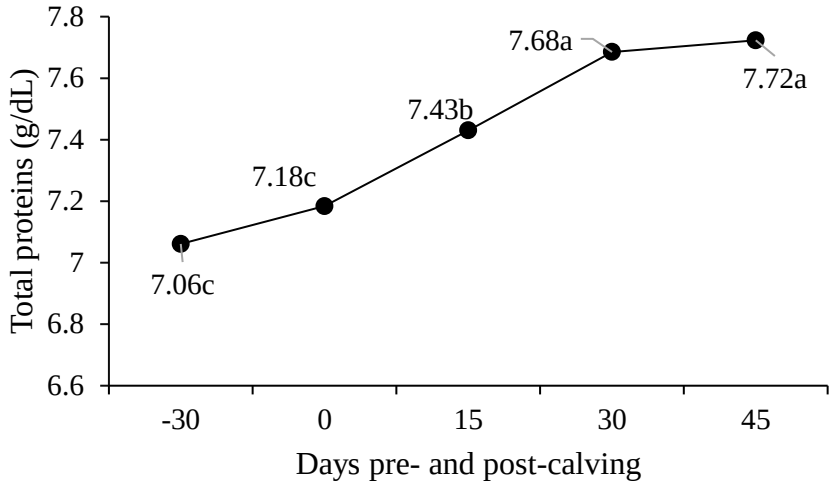
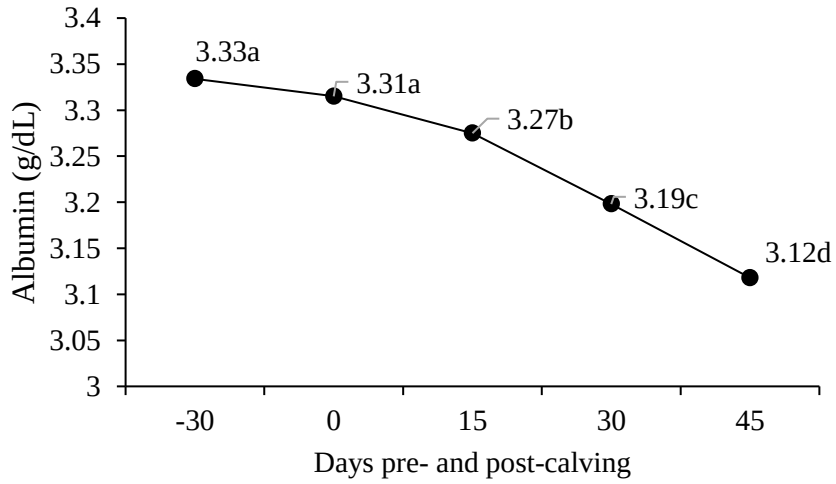


Figure 5. HDL (a), LDL(b) and VLDL(c) serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentrations (P<0.10).

(a)



(b)



(c)

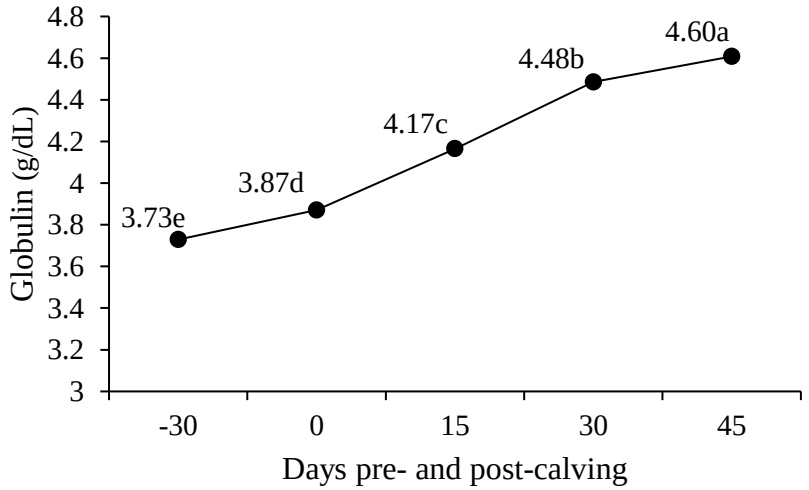


Figure 6. Total protein (a), albumin (b) and globulin (c) serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentrations (P<0.10).

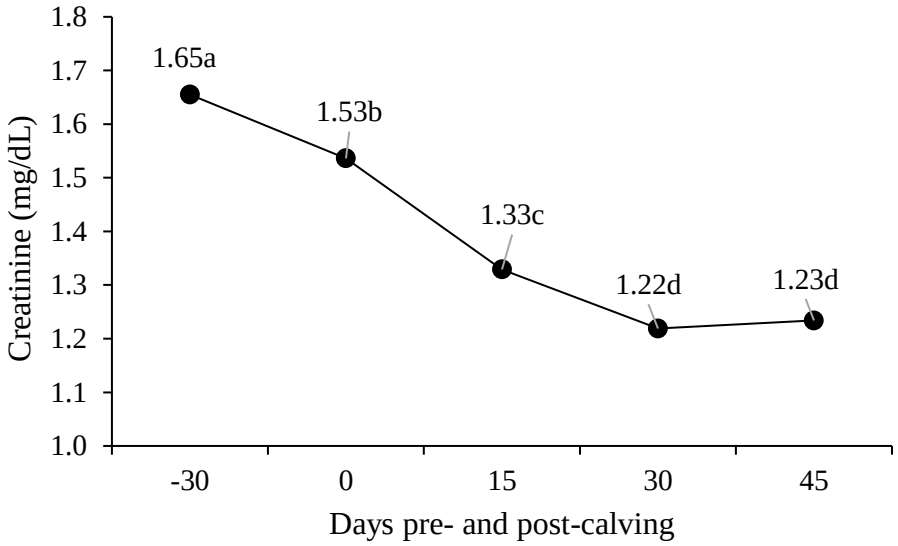


Figure 7. Creatinine serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentration (P<0.10).

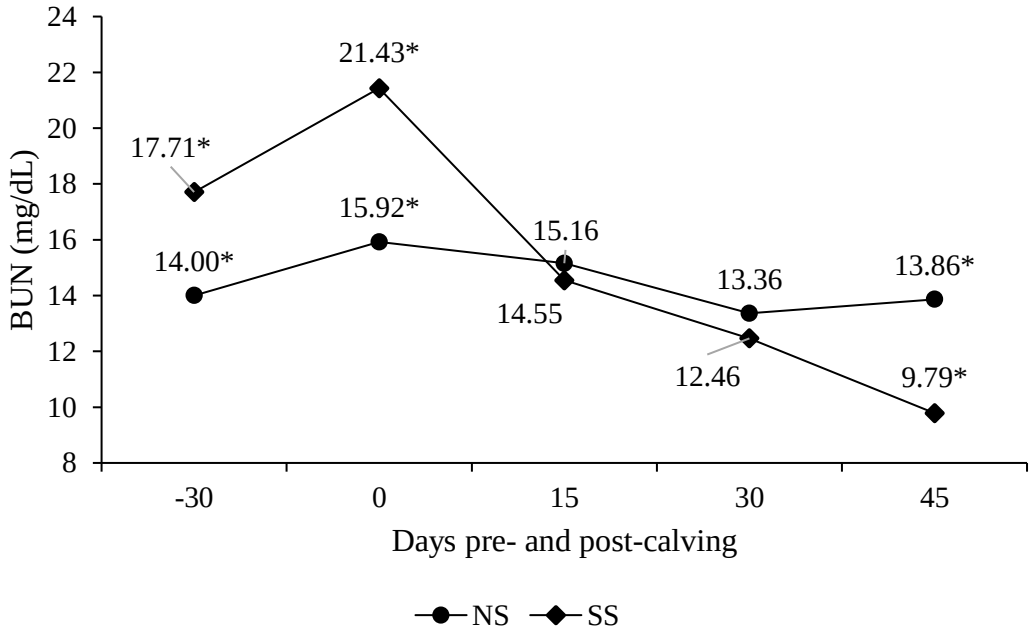


Figure 8. BUN concentrations during pre- and post-calving. Numbers followed by (*) are significantly different between treatments (P<0.10).

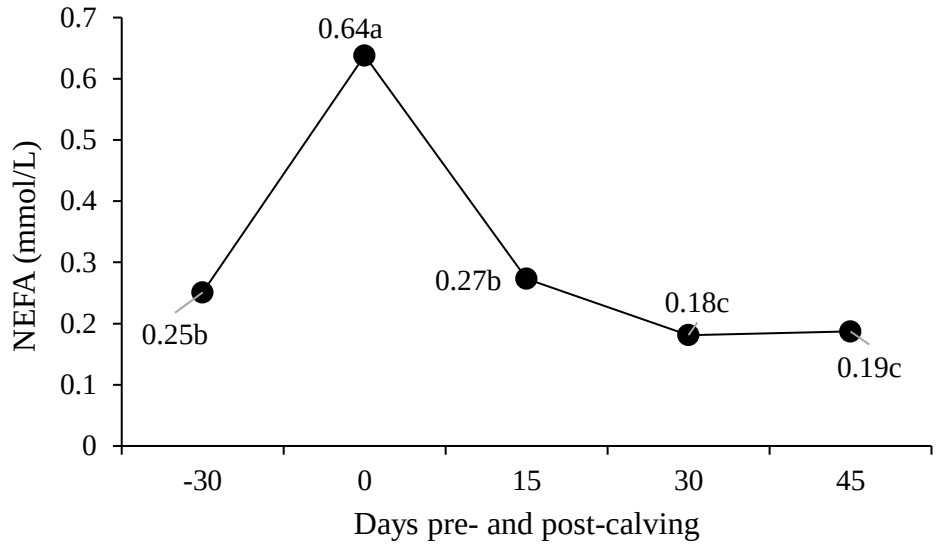


Figure 9. Non-esterified fatty acids (NEFA) serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentration (P<0.10).

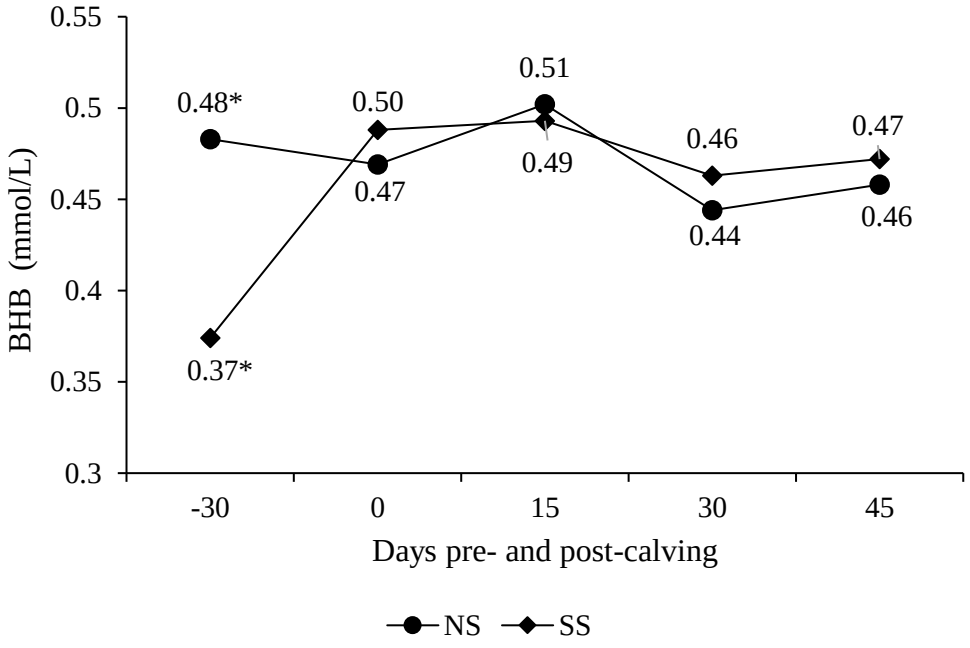
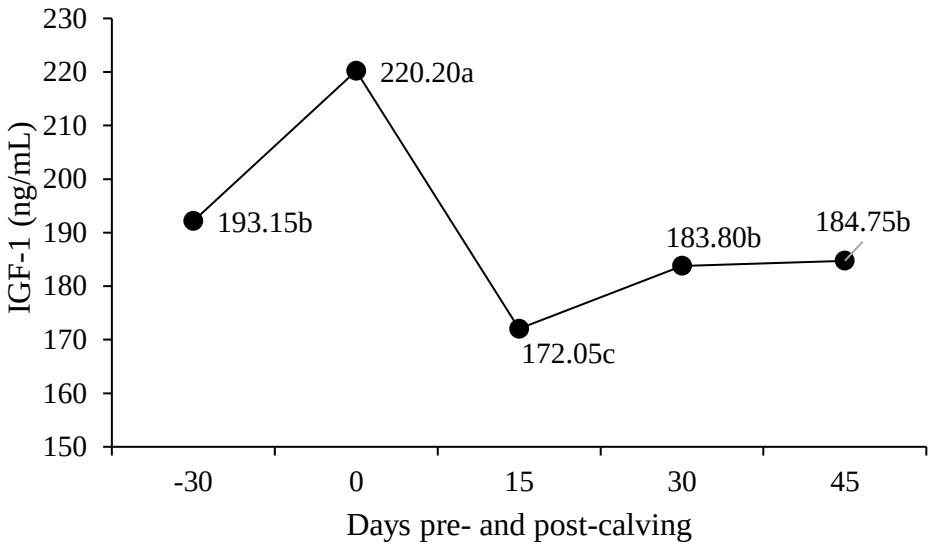


Figure 10. β-hydroxybutyrate (BHB) serum concentrations during pre- and post-calving. Numbers followed by (*) are significantly different between treatments (P<0.10).

(a)



(b)

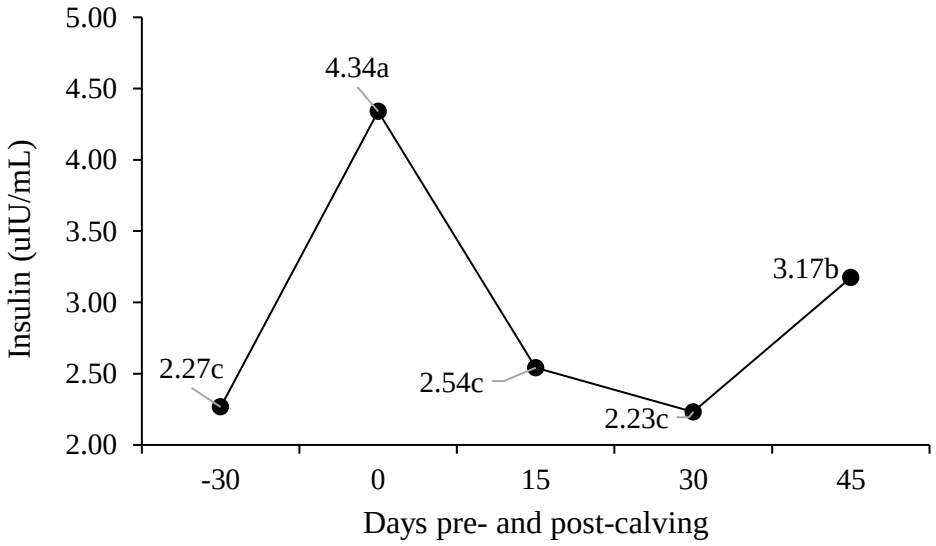
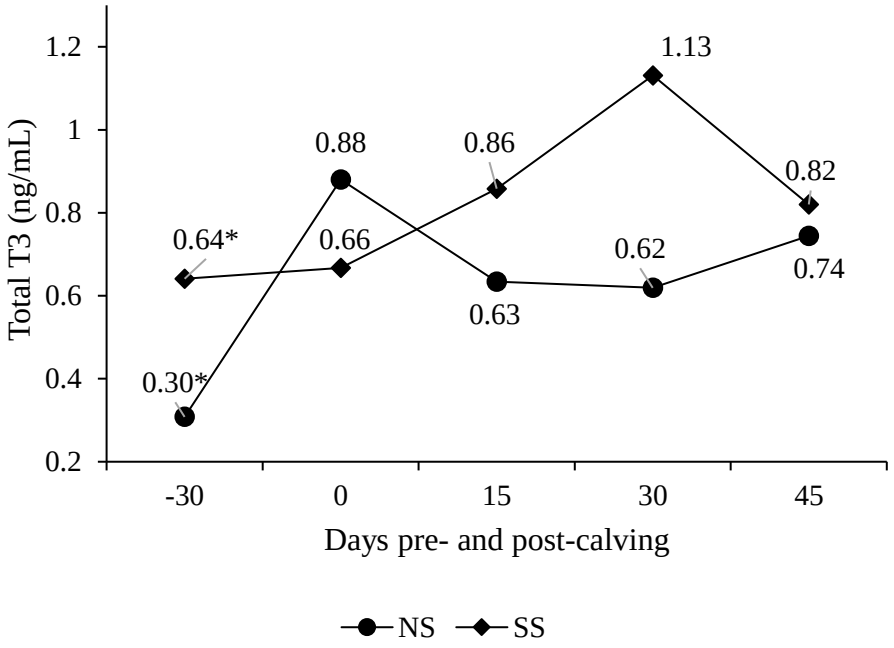


Figure 11. IGF-1 (a) e Insulin (b) serum concentrations during pre- and post-calving. Different letters declare significantly different serum concentration (P<0.10).

(a)



(b)

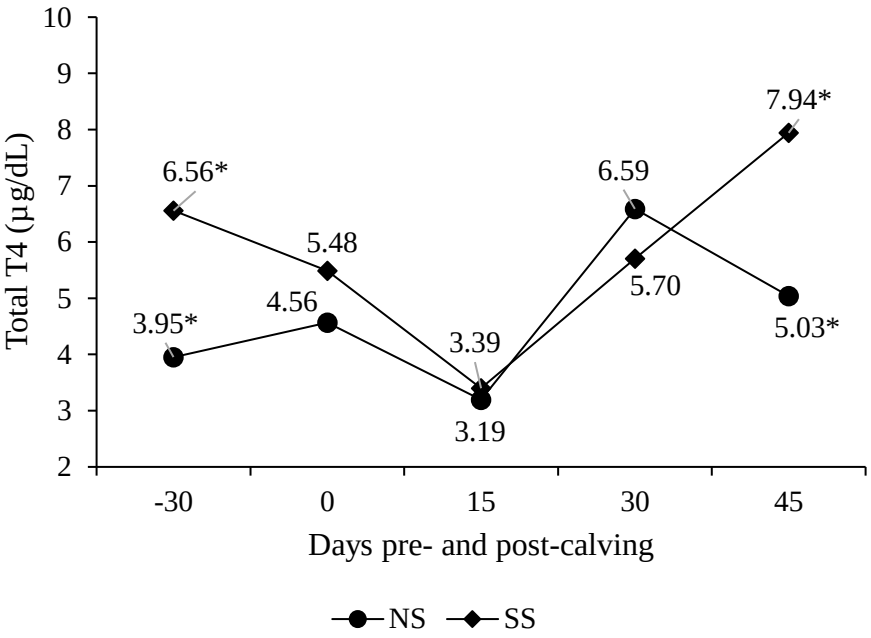


Figure 12. Total T3 (a) and T4 (b) serum concentrations during pre- and post-calving. Numbers followed by (*) are significantly different between treatments (P<0.10).