

JAVIER ENRIQUE GARCÉS CARDENAS

**NUTRITIONAL AND METABOLIC EVALUATION OF NELLORE COWS
SUPPLEMENTED OR NOT DURING THE PERIPARTUM**

Thesis submitted to the Animal
Science Graduate Program of the
Universidade Federal de Viçosa in
partial fulfillment of the requirements
for the degree of *Doctor Scientiae*.

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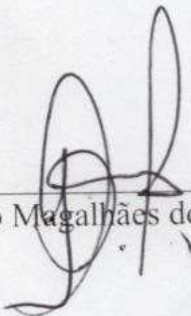
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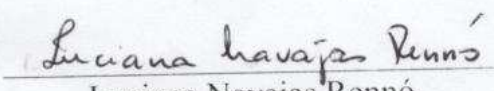
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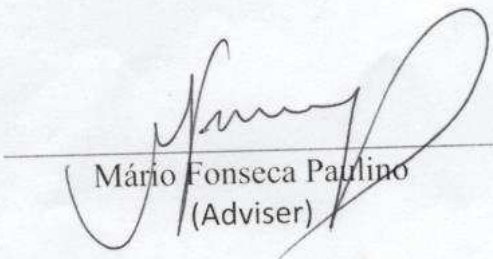
Eriton Egídio Lisboa Valente



Edenio Detmann



Luciana Navajas Rennó



Mário Fonseca Paulino
(Adviser)

“Plant your garden and decorate your soul, rather than waiting for someone to bring you flowers. And you learn that you really can stand, that you really are strong, and that you can go much further after thinking you cannot anymore. And that really a life has value and that you have value of life!”

William Shakespeare

To God, omniscient and companion of my work

I offer.

To my relatives

I dedicate.

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BIOGRAPHY

JAVIER ENRIQUE GARCÉS CARDENAS, son of Oscar de Jesus Garcés Moncada and Rocio Del Socorro Cardenas Cardenas, was born in Támenesis, Antioquia – Colombia on February 08, 1985.

He began his undergrad education in Veterinary Medicine and Animal Science at Universidad de Cordoba in Monteria, Cordoba, Colombia, and he successfully graduated in June, 2011.

Moreover, in August of 2011, he started the Master Science program in Animal Science at Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil, with major in Ruminant Production and Nutrition. He obtained the degree of Magister Scientiae in Animal Science in February, 2013. In March of the same year, he started his doctorate program in Animal Science with major in Ruminant Production and Nutrition at the Universidade Federal de Viçosa.

ABSTRACT

GARCES CARDENAS, Javier Enrique, D.Sc., Universidade Federal de Viçosa, February, 2017. **Nutritional and metabolic evaluation of nellore cows supplemented or not during the peripartum.** Adviser: Mário Fonseca Paulino.

The objective of this study was to evaluate the effect of supplementation during pre- and/or post-calving on performance, nutritional and metabolic characteristics of Nellore cows grazing *Brachiaria decumbens*. The experiment lasted 120 days from July to November, during dry season and dry – to – rainy transition period. For the experiment, 48 multiparous Nellore cows were used, with an average aged of 6 years. The average body weight (BW) and body condition score (BCS) at the beginning of the experimental period was 501 ± 55 kg and 5.4 ± 0.65 , respectively. The experiment was carried out according to a completely randomized design, following a 2×2 factorial arrangement, where two supplementation strategies were tested, supplementation (1 kg/d of 20% of crude protein, CP) and no supplementation, during two periods, pre- and post-calving. Therefore, there were four treatments: supplementation during pre- and post-calving (PrePost), supplementation during pre-calving but not during post-calving (Pre), no supplementation during pre-calving but during post-calving (Post), and no supplementation during pre- or post-calving (Control). During the pre-calving, supplemented cows showed a higher intake of dry matter (DM), organic matter (OM), and CP, compared to cows without supplementation ($P < 0.10$). However, supplementation during pre-calving did not affect forage intake ($P > 0.10$), neither digestibility of neutral detergent fiber. Supplementation during post-calving showed an effect on intake of DM and OM when expressed as g/kg of BW ($P < 0.10$). Supplementation during pre- and/or post-calving did not affect BW, average daily gain, BCS, back fat, calves birth weight and calves BW at 60 days old ($P > 0.10$). Supplementation during pre- and/or post-calving did not affect ($P > 0.10$) serum concentration of glucose, total proteins, albumin, globulins, blood urea nitrogen (BUN), non-esterified fatty acids (NEFA) and β -hydroxybutyrate (β HB). Similarly, there were no significant interaction between treatments and time when these variables were measured ($P > 0.10$). However, the concentration of these variables changed significantly depending of the sampling day ($P < 0.10$). In conclusion, that 1 kg/d of 20% of CP supplement for Nellore cows grazing low quality tropical pasture during pre- and/or post-calving on is not enough to improve the performance, nutritional and

metabolic characteristics, as well as, there is no carry-over effects of pre-calving supplementation on response post-calving.

RESUMO

GARCES CARDENAS, Javier Enrique, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Avaliação nutricional e metabólica de vacas nelore suplementadas ou não no periparto.** Orientador: Mário Fonseca Paulino.

O objetivo deste estudo foi avaliar o efeito da suplementação durante pré e/ou pós-parto no desempenho, características nutricionais e metabólicas das vacas Nelore pastejando *Brachiaria decumbens*. O experimento foi conduzido durante 120 dias, de julho a novembro, durante a estação seca e o período de transição entre seco e chuvoso. Para o experimento, foram utilizadas 48 vacas Nelore múltiparas, com idade média de 6 anos. O peso corporal médio (PC) e escore de condição corporal (ECC) no início do experimento foram de 501 ± 55 kg e $5,4 \pm 0,65$, respectivamente. O experimento foi realizado de acordo com um delineamento inteiramente casualizado, seguindo um esquema fatorial 2×2 , onde duas estratégias de suplementação foram testadas, suplementação (1 kg/d com 20% de proteína bruta, PB) e sem suplementação, durante dois períodos, pré e pós-parto. Portanto, houve quatro tratamentos: suplementação durante pré e pós-parto (PrePost), suplementação durante pré-parto, mas não durante pós-parto (Pré), sem suplementação durante pré-parto, mas durante pós-parto (Pós) e sem suplementação durante pré ou pós-parto (Controle). Durante o pré-parto, as vacas suplementadas apresentaram maior consumo de matéria seca (MS), matéria orgânica (MO) e PB, em comparação com as vacas sem suplementação ($P < 0,10$). No entanto, a suplementação durante o pré-parto não afetou a ingestão de forragem ($P > 0,10$), nem a digestibilidade da fibra em detergente neutro. A suplementação durante o pós-parto mostrou um efeito na ingestão de MS e MO quando expresso em g/kg de PC ($P < 0,10$). A suplementação durante pré e/ou pós-parto não afetou PC, ganho médio diário, ECC, gordura da garupa, peso do bezerro ao nascimento e PC dos bezerros aos 60 dias de idade ($P > 0,10$). A suplementação durante pré e/ou pós-parto não afetou ($P > 0,10$) a concentração sérica de glicose, proteínas totais, albumina, globulinas, nitrogênio ureico no sangue (BUN), ácidos graxos não esterificados (NEFA) e β -hidroxibutirato (β HB). Da mesma forma, não houve interação significativa entre tratamentos e tempo em que essas variáveis foram medidas ($P > 0,10$). No entanto, a concentração dessas variáveis mudou significativamente dependendo do dia da amostragem ($P < 0,10$). Em conclusão, a suplementação de 1 kg/d com 20% PB para vacas Nelore que pastoreiam pastagens tropicais de baixa qualidade durante pré e/ou pós-parto

não é suficiente para melhorar o desempenho, as características nutricionais e metabólicas, bem como, não há efeitos da suplementação pré-parto na resposta pós-parto.

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Introduction

In the beef production systems in Brazil, 99% of the production comes from pasture, presenting the advantage of providing low cost of production and high practicality (Paulino et al., 2008). It is important to consider that the practice of supplementation in beef cattle can contribute to the stability of the productive system (Fernandes et al., 2012). Supplementation of beef cows during pre- and/or post-calving periods can be an alternative for improving the efficiency of grazing systems in the tropical, especially when protein supplements are used. Protein supplementation can improve the activity of the rumen microbiota and fiber degradation, allowing a better utilization of forages, especially during the dry season (Valente et al., 2012; Detmann et al., 2014a).

Production efficiency of beef cows can be improved by a better understanding of the production cycle, and by paying attention to the nutritional requirements of the cows. Therefore, there is the need to develop nutritional and feeding strategies able to guarantee the stability of the production system. Most of nutritional studies have mainly focused on growing and fattening beef cattle; therefore, there is a lack of information about nutritional management of beef cows during peripartum (Shoup et al., 2015). From the few studies on supplementation of zebu cows during peripartum the results are contradicting, as showing no response (Lopes et al., 2016b) or showing improvement of cows performance (Godoy et al., 2004).

The development feeding strategies for beef cows is facilitated when there is a basic understanding of the production cycle and the nutrients required, so that recommendations can be made on the appropriate use of supplementation levels. However, the use of low-level supplementation in quantity or quality should be practiced with caution, since cow management in the peripartum depends on the previous use of a management system capable of coordinating animal-environment interaction in the search for greater efficiency (Mulliniks et al., 2016).

In addition, the lactation period is usually conducted in areas of lower quality pastures and the supplementation of the beef cows is still very little used in Brazil. Considering management models in which the calf are supplemented using creep-feeding, consistent results in the literature have demonstrated that this management practice does not alter the productive performance of the lactating matrices (Barros et al., 2014; Lopes

et al., 2016a). Therefore, it is necessary to change the focus of the supplementary management when the objective is to increase the productive efficiency of the matrices.

Currently, one of the most important goals for the beef cattle production system is to establish nutritional management programs based on the formulation of diets to increase the reproductive efficiency of the matrices, which is dictated by the return of ovarian activity after calving (Emerick et al., 2009; Neves et al., 2010).

On the other hand, blood metabolites have been used to indicate metabolic disorders that occur during post-calving in cows, and can also serve as indicators of nutritional status. Pre- and/or post-calving supplementation may contribute to decrease the effects of negative energy balance (NEB), which is usually indicated by increased plasma glucose concentration and decreased concentrations of non-esterified fatty acids (NEFA) and β -hydroxybutyrate (β HB) (Silveira et al., 2012). The magnitude of NEB will depend on the body condition of the cow at calving and post-calving nutritional management (Roche et al., 2000). Changes in glucose concentrations, NEFA and β HB, which are indicators of energy availability and the metabolic status of the animal, can be used to estimate the magnitude of NEB (Vizcarra et al., 1998).

Thus, the two following hypothesis have been raised: 1) supplementation during pre- and/or post-calving can improve the nutritional and productive performance of beef cows; 2) supplementation during peripartum can improve metabolic and nutritional status of Nellore cows.

The objective of this study was to evaluate the effect of supplementation during pre- and/or post-calving on performance, nutritional and metabolic characteristics of Nellore cows grazing *Brachiaria decumbens*.

Materials and methods

Ethics statement

All procedures in this study were approved by the Ethic Commission in Use of Production Animals of the Universidade Federal de Viçosa, protocol N° 19/2015.

Experimental design, animals, and treatments

This experiment was carried out at the Beef Cattle Section of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20°45' S and 42°52' W). The experimental area is located on a hilly area at an altitude of 670 m and with an average annual rainfall of 1300 mm. The experiment lasted 120 days from July to November, during dry season and dry – to – rainy transition period. It was divided in two phases: 1) 60 days before calving (pre-calving period); and 2) 60 days after calving (post-calving period).

For this experiment, 48 multiparous Nellore cows were used, that were at an average 6 years of age. The average body weight (BW) and body condition score (BCS, 1-9) at the beginning of the experimental period was 501±55 kg and 5.4±0.65, respectively. At the beginning of the experiment all cows were in the 6 month of pregnancy, the calving of all cows occurred with a maximum difference of 18 days.

The experiment was carried out according to a completely randomized design, following a 2 × 2 factorial arrangement, where two supplementation strategies were tested, supplementation and no supplementation, during two periods, pre- and post-calving. Therefore, there were four treatments: supplementation during pre- and post-calving (PrePost), supplementation during pre-calving but not during post-calving (Pre), no supplementation during pre-calving but during post-calving (Post), and no supplementation during pre- or post-calving (Control).

Eight paddocks were used during pre-calving (2 paddocks/treatment), with a total area of 39 hectares. After calving, cows were relocated to other 8 paddocks (2 paddocks /treatment) in an area of 70 hectares. Both areas were with *Brachiaria decumbens*, provided with covered feeders and drinkers. The treatments were distributed to cows in a completely randomized design, where the paddocks were considered the experimental units.

The cows in treatments PrePost, Pre, and Post received daily the amount of 1 kg/cow/day of supplement, when they were supplemented at 10h00 and provided in group feeders. The supplement was formulated using the feed composition information provided by CQBAL 3.0 to present 200 g/kg of crude protein (CP) in dry matter (DM) basis (Table 1). The supplement composition was: corn (340 g/kg), wheat bran (340 g/kg), soybean meal (240 g/kg), mineral premix (80 g/kg). All cows had ad libitum access to a mineral premix during all time. The premix composition was: dicalcium phosphate (500 g/kg), sodium chloride (472 g/kg), zinc sulfate (15 g/kg), copper sulfate (7 g/kg), cobalt sulfate (0.5 g/kg), potassium iodate (0.5 g/kg), and manganese sulfate (5 g/kg).

The cows were weighted at different moments during the experiment, and the weighting was always performed at 6h30. During pre-calving, cows were weighted at the start of the period (BW_{pre_start}) and 15 days before calving (BW_{pre_15d}). Then, the BW at calving ($BW_{calving}$) was calculated as:

$$BW_{calving} = BW_{pre_15d} + ADG_{pre} \times \text{days to calving}$$

where, ADG_{pre} is the average daily gain in the pre-calving period.

During post-calving, cows were weighted five days after calving (BW_{post_5d}) and at the end of the post-calving period (BW_{post_end}). The weighting procedure consisted of the cows being scored for body condition (i.e., BCS_{pre_start} , $BCS_{calving}$, BCS_{post_start} , and BCS_{post_end}) (scale 1 to 9) according to the National Research Council (NRC, 1996), and subcutaneous fat thickness on the back (i.e., $EGP8_{pre_start}$, $EGP8_{calving}$, and $EGP8_{post_end}$), which was measure using an ultrasound instrument (Aloka SSD 500, Hitachi). Calves were weighted at birth (CBW) and 60 days after (BW_{calf_60d}).

Forage sampling

To evaluate the unfritional quality of the forage, every 30 days grass samples were collected by hand plucked sampling. Also, samples were collected cutting at ground level of four 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 weighted, oven-dried (55°C), and then ground to pass through a 1 and 2 mm screen in Wiley mill (model 3, Arthur H. Thomas, Philadelphia, PA).

Intake and digestibility assay

The purpose of the two trials were to evaluate intake and digestibility during pre- and post-calving, with a duration of 9 days each one. The first trial was performed 30 days after the beginning of the experiment and the second trial was performed during post calving period, 20 days after the last calving.

Six of the nine days of the assay were destined to adaptation of the animals to the chromium oxide (Cr_2O_3) and titanium dioxide (TiO_2), the animals received the marker during the first 8 days at 10h00. The Cr_2O_3 used to estimate fecal excretion, was packaged in paper cartridges in the amount of 15 g per animal/day for cows and was directly introduced into the esophagus by using a rubber tube; while the TiO_2 used to estimate supplement individual intake was mixed with the supplement distributed to the cows in an amount equal to 20 g per animal/day. Starting at day 6 of the trial, feces were sampled at: 17h00 at day 6, 15h00 at day 7, 10h00 at day 8, and 7h00 at day 9. Feces were sampled immediately after defecation or directly from the rectum in quantities of approximately 300 g. Feces samples were over-dried (55°C). A quantity of 25 g from each of the 4 days proportionally subsampled into a composite sample, and then ground to pass through a 1 and 2 mm screen in Wiley mill.

At day 5 of the trial, it was performed a hand plucked sampling at each paddock. These samples were used to estimate voluntary dry matter intake and digestibility of the forage.

To end, the last day of the trial, spot urine samples (10 mL) were collected from spontaneous micturition 4 hours after the supplement was offered. Urine samples were diluted in 40 mL of H_2SO_4 (0.036 N) and frozen (-20°C). These samples were used to evaluate the microbial protein production and urinary urea nitrogen of cows.

Blood sampling

Taking calving day as Day 0, blood samples were collected just before supplementation on days -60, -30, -15, 0, 14, 28, 35, 42, 49 and 56. As an illustration, blood samples were collected by jugular vein puncture, using vacuum tubes with separator gel (Vacuplast®), to quantify urea, total protein, albumin, non-esterified fatty acid (NEFA), β -hydroxybutyrate (βHB), and progesterone contents; and using a 4 mL tube with fluoride anticoagulant and K_3EDTA (Labor IMPORT) to quantify plasma concentration of glucose. After collected, the samples were centrifuged at $3600 \times g$ for 15

min. In effect, serum and plasma were immediately frozen at -20°C in duplicates until further analysis.

Chemical analyses and calculations

Forage, feces, and supplement samples 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 dry matter (DM; INCT-CA method G-003/1), ash (INCT-CA method M-001/1), crude protein (CP; INCT-CA method N-001/1), ether extract (EE; INCT-CA method G-004/1), neutral detergent fiber corrected for ashes and protein (NDFap; INCT-CA method F-002/1), neutral detergent insoluble nitrogen (NDIN). To further explain, indigestible neutral detergent fiber (iNDF; INCT-CA method F-009/1) was quantified by *in situ* incubation procedures with non-woven textile bags (100 g/m²) for 288 hours in samples processed at 2 mm. In addition, fecal samples were evaluated for the contents of chromium (INCT-CA method M-005/1) and titanium (INCT-CA method M-007/1).

In addition, potentially digestible dry matter (pdDM) was estimated using samples of forage availability, following Paulino *et al.* (2008) equation:

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

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 quantity of Cr₂O₃ offered and the marker concentration in feces. Individual supplement intake (ISI) in DM base was estimated as a ratio of the TiO₂ excreted in feces and marker concentration in the supplement.

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:

$$\text{DMF} = \left[(\text{FE} \times \text{iNDF}_{\text{feces}}) - \text{ISI} \times \text{iNDF}_{\text{sup}} \right] \div \text{iNDF}_{\text{forage}}$$

where, EF = fecal excretion (kg/d), iNDF_{feces} = indigestible neutral detergent fiber in feces (kg/kg), ISI = individual supplement DM intake (kg/d), iNDF_{sup} = indigestible neutral detergent fiber in supplement (kg/kg), and iNDF_{forage} = indigestible neutral detergent fiber in forage (kg/kg).

Furthermore, urine and blood analyses were performed in the Laboratory of Animal Physiology and Reproduction, Department of Animal Science, Universidade Federal de Viçosa. The following kits were used for the analyses: creatinine (K067), uric acid (K139), urea (K056), total proteins (K031), albumin (K040) and glucose (K082) from Bioclin® Quibasa (Belo Horizonte, Brazil). NEFA (FA115) and β HB (RB1007) were measured from all the blood samples, except from the blood samples collected on day -15, from Randox® (Crumlin, Antrim, United Kingdom) and using the automatic biochemical analyzer (Mindray, BS200E model). Blood urea nitrogen (BUN) was estimated as 46.67% of total blood urea. Progesterone was analyzed by chemiluminescent from the samples collected on days 35, 42, 49 and 56 post-calving, using Access® 2 with the kit BK33550 from Beckman Coulter® (Brea, USA). Allantoin was analyzed by the colorimetric method as described by Chen and Gomes (1992).

An estimate was made of 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 from the spot urine samples and shrunk body weight (SBW), calculated using the equation proposed by Costa e Silva *et al.* (2012):

$$CE(\text{g/d}) = 0.0345 \times \text{SBW}^{0.9491}$$

where, SBW was estimates as $0.8800 \times \text{BW}^{1.0175}$ according to Costa e Silva *et al.* (2016).

Total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in urine. Ruminal synthesis of microbial nitrogen (NMic) was calculated as a function of absorbed purines using the equations proposed by Barbosa *et al.* (2011):

$$\text{AP} = \frac{\text{DP} - 0.301 \times \text{BW}^{0.75}}{0.80}$$

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

$$\text{NMic} = \frac{(70 \times \text{AP})}{(0.93 \times 1000 \times 0.137)}$$

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

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:

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

where, μ = overall mean; T_i = fixed effect of treatment; $\delta_{(i)j}$ = random effect of paddock j within the treatment i ; $\epsilon_{(ij)k}$ = random error effect associated to observation l nested in paddock k , which is assumed to be iid $\sim N(0, \sigma^2)$.

For the purpose of the variables measured during pre-calving, including the variables from the digestibility trial, the effects of treatments were evaluated by a simple comparison between supplemented and not supplemented cows. For the variables measured during post-calving, the sums of squares of treatments were decomposed in orthogonal contrasts, to test interaction and independent effects of supplementation pre- and post-calving, following a 2×2 factorial arrangement.

The ANOVA for metabolites were performed by considering the repeated measures, where the best (co) variances structures was chosen using the Akaike's Information Criterion.

All were performed using the PROC MIXED in SAS 9.4 (Inst. Inc., Cary, NC). When necessary, the means of treatments 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.

Results

The area used for grazing presented an average availability of DM and pdDM of 4.12 ± 0.358 t/ha and 2.36 ± 0.131 t/ha for the pre-calving period, respectively. On the other hand, during post-calving, the area used for grazing presented an average availability of DM and pdDM of 4.68 ± 0.325 t/ha and 2.69 ± 0.125 t/ha, respectively. The forage samples collected by the hand-plucked method had an average 51 and 68 g/kg of CP in a DM base (Table 1), during the pre and post-calving period, respectively.

In the pre-calving period, supplemented cows showed a higher intake kg/day of DM, OM, and CP ($P < 0.10$), compared to cows without supplement. However, when expressed as g/kg BW, there was no difference ($P > 0.10$) between supplemented and not supplemented cows (Table 2). There was no effect of supplementation ($P > 0.10$) on intake of DMF, NDFap, iNDF, digested organic matter (dOM), and digestible neutral detergent fiber.

Equally important, the supplementation during pre-calving did not affect ($P > 0.10$) digestibility of OM, CP, and NDFap. Similarly, supplementation did not affect ($P > 0.10$) the synthesis of nitrogen compounds, dOM, NMic, and NMic/N intake (NMic/NI), and neither the efficiency for synthesis of microbial protein (Emic, g CP/kg dOM) (Table 3). Similar behavior was observed for the BW, average daily gain (ADG), BCS, EGP8 and calves birth weight (Table 4).

As supplementation continued during the pre-calving period, it had no affect ($P > 0.10$) towards the serum concentration of glucose, total proteins, albumin, globulins, and BUN, observed on samples collected on days -60, -30, -15 and 0 (Table 5). Similarly, there was no interaction ($P > 0.10$) between treatments and time when these variables were measured. However, the concentration of these variables changed significantly ($P < 0.10$) depending of the calving day. For glucose (Figure 1), higher serum concentrations were observed at calving (day 0), followed by day -60, -15, and -30 ($P < 0.10$). For total proteins (Figure 2), serum concentrations were similar at day -60 and -30 ($P > 0.10$), but higher than at day -15 and at calving ($P < 0.10$). Likewise, at day -15 presented the highest serum protein concentration in relation to the day of delivery ($P < 0.10$). For serum albumin (Figure 2), similar serum concentrations were observed on days -30, -15 and day of delivery ($P > 0.10$), however, these were higher than day -60 ($P < 0.10$). For globulins (Figure 2), higher serum concentrations were observed at day -15 compared to day of calving. Similarly, at day -15 had a higher serum globulin concentration in relation to the day of delivery. For BUN (Figure 3), serum concentrations were similar at day -30, -15 and at calving ($P > 0.10$), but higher than at day -60 ($P < 0.10$).

Serum concentrations of NEFA and β HB were not affected ($P > 0.10$) by the supplementation during pre-calving (Table 5). Moreover, there was no interaction ($P > 0.10$) between treatments and time. For β HB (Figure 4), the concentration did not change ($P > 0.10$) depending of the calving day. However, for NEFA, the concentration changed ($P < 0.10$), which all depended on the calving day, where concentrations during day -30 and calving were similar, but higher than day -60.

Similarly, supplementation during pre- and/or post-calving period did not affect ($P>0.10$) intake after calving (kg/d) of DM, DMF, OM, CP, NDFap, iNDF, dOM, and dNDF (Table 6). However, supplementation during post-calving period showed an effect on DM and OM intakes when expressed as g/kg BW ($P<0.10$).

Supplementation during post-calving period did not affect ($P>0.10$) the coefficient of digestibility of OM and NDFap, neither had an effect on the diet concentration of dOM, in the production of NMic, or in the ratio NMic/NI. However, supplementation during post-calving period increased the coefficient of digestibility of CP (Table 7). Additionally, supplementation during pre- and/or post-calving period did not affect ($P>0.10$) the variables of performance measured at the end of the post-calving period: BW, ADG, BCS, EGP8, and calf BW (Table 8).

Similar to the other results, supplementation during pre- and/or post-calving period did not affect ($P>0.10$) serum concentration of glucose, total proteins, albumin, globulins, and BUN, observed on samples collected on days 14, 28, 35, 42, 49, and 56. Therefore, there was no significant interaction ($P>0.10$) between treatments and time when these variables were measured (Table 9). However, the concentration of these variables changed significantly ($P<0.10$) depending on the calving day. For glucose (Figure 1), serum concentrations were similar ($P>0.10$) to days 49 and 56 but higher than 42, 14, 28, and 35 days. On day 35 the lowest ($P<0.10$) level was observed. For total proteins (Figure 2), serum concentrations were similar ($P>0.10$) at day 14, 35, 42 and 56, but higher than at day 28 and 49, except for day 56 that was similar to day 28, also day 28 and 49 were similar. For albumin (Figure 2), serum concentrations were similar at day 14, 35, and 42, but higher than at day 28, 49, and 56. Albumin concentration in serum during days 28, 49, and 56 were similar among each other. Moving on to globulins (Figure 2), serum concentrations were similar ($P>0.10$) at day 14, 28, 35, 42, and 56, except day 49 ($P<0.10$) which was similar ($P>0.10$) to the days 14, 28, and 42, however smaller than to the days 35 and 56. For BUN (Figure 3), serum concentrations were similar ($P>0.10$) at days 14 and 28, but lower than all other days, days 35 and 42 were similar to each other. On days 49 and 56 the serum concentrations were different ($P<0.10$).

Serum concentrations of NEFA and β HB during post-calving were not affected ($P>0.10$) by supplementation during pre- and/or post-calving (Table 9). Moreover, interaction between treatments and time was not significant ($P>0.10$). However, the concentration for both variables changed ($P<0.10$) depending of the calving day. For NEFA (Figure 4), serum concentration were similar ($P>0.10$) at day 14, 28 and 35, but

higher than at days 42, 49 and 56, except for day 35 that was similar ($P>0.10$) to day 42. Serum concentration of NEFA at day 42 was higher than at day 49 and 56. Serum concentration of NEFA at day 49 was higher than at day 56. For β HB (Figure 4), serum concentrations was higher ($P<0.10$) at day 14 than at days 28, 35, 42, and 49. Concentrations at day 28, 35, and 42 were similar ($P>0.10$) among each other, and higher ($P<0.10$) than at days 49 and 56, which were similar ($P>0.10$) between them.

Progesterone concentration in blood did not differ ($P>0.10$) among treatments, and no significant interaction ($P>0.10$) between treatments and time was observed. However, the concentration changed ($P<0.10$) depending of the calving day (Table 9). For progesterone (Figure 5), serum concentration were similar ($P>0.10$) at day 35, 42 and 49. On day 56 were higher ($P<0.10$) than at days 35 and 42, but similar ($P>0.10$) at day 49.

Discussion

Forage is the most relevant input for beef production in tropical areas. However, not all the DM present in the forage and consumed by the cow is really used, the pdDM it incorporates information of available fraction for use and a grazing efficiency, being a better estimator of feed availability for cattle in grazing than the DM of forage. Availability of pdDM of the forage integrates quality and quantity, independent to the season, therefore it is necessary to understand all the components of the system (i.e., animal, forage, and environment) and the interactions among them (Paulino et al., 2004).

It is important to consider supplementation as a contributor to the stability of the production system (Fernandes et al., 2012). But it is also important to realize that the results of lowquality and quantity supplementation depend on the management system used previously (Mulliniks et al., 2016).

According to Detmann et al. (2014a; 2014b), better fiber degradation has been observed when increasing the levels of CP in the diet to around 100 g/kg of DM. Also, voluntary intake of forage was increased with CP concentrations close to 145 g/kg of DM. Average content of CP in the forage during the experiment was approximately 56 g/kg of DM (Table 1), showing that there was a restriction on protein compared to the recommended levels. Supplementation of 1 kg with 20% CP, the CP level in the diet increased to 80 g/kg of DM, being lower than the suggested level.

Results obtained in tropical conditions, showed that extra supplied of nitrogen compounds to cows grazing low quality forage, favors growth of fibrolytic bacteria, which improves ruminal degradation of NDF, voluntary intake of forage, and energy extracted from fiber (Paulino et al., 2008). However, in our study, no effect was observed on digestibility of NDF (Table 3 and 6). Fortunately, our results can indicate that the quantity of CP included in the supplement was not enough to cause the positive effect voluntary intake of forage observed in other studies. With the results obtained, it is important to recommend for low quality conditions in the pasture and with a quantity of 1 kg/d, supplement with a minimum of 40% CP.

Although supplementation did not affect performance and body condition, supplemented cows had the tendency to become heavier at calving and give birth to heavier calves. These differences could indicate a benefit of supplementation during pre-calving, improving nutrients supply, mainly protein and energy. In addition to taking into account that this difference may not have been significant because of the number of animals used in each treatment. These results corroborate with Godoy et al. (2004), who also found heavier BW at calving when cows received a supplementation of 1 kg/animal/d containing 16% of CP in DM basis.

Results measured at post-calving indicated that supplementation during pre- and/or post-calving did not affect intake and digestibility, productive performance. Similarly, to this study, Lopes et al (2016b) evaluated a supplementation of 1 kg/animal/d containing 32% of CP in DM basis, in beef cows in a grazing system during post-calving, concluding that supplementation does not affect nutritional and productive performance. On the other hand, Godoy et al., (2004) observed heavier BW and higher BSC at 112 days after calving, when cows were supplemented with 1 kg/animal/d containing 16% of CP in DM basis. Contradictory results as the above mentioned, show the necessity of more studies about the effect of supplementation during pre- or/and post-calving on nutritional and productive performance of beef cows.

Supplementation during post-calving increased intake of DM and OM in g/kg of BW (Table 5). An important remark is the absence of a substitution effect between the forage and the supplement, which is interesting because the idea is to better use the forage through an associative effect as stimulus on intake. Also, supplementation during post-calving increased apparent digestibility of CP (Table 6), These increments could be due to the supplementation, possibly the result of the lower proportion of metabolic fecal fraction in relation to ingested nutrients (Barros et al., 2011). However, this increment did

not increase forage intake, confirming the results from Figueiras et al. (2015), where protein supplementation for cattle grazing during transition from dry to rainy season in tropical areas did not affect voluntary intake of forage.

In the present study, were observed a decrease of glucose concentration in serum at the end of the gestation (Figure 1) was observed. The decrease of blood glucose level with the progression of the gestation could be related to fetal demand (Sletmoen-Olson et al. 2000), as well as a compensation observed during calving, which can be explained by the stimulus of the glycogenesis paths for minimizing the stress during calving (Collier, 1985). After calving and until 35 days post-calving, a decreased in glucose was also observed. This decrement can be explained by the higher energy demand for milk production, where the glucose is taken from the blood by the mammal gland to produce lactose (Larson, 1985). On days 49 and 56 serum levels of glycoside were restored.

In order to evaluate protein metabolism, the blood levels of total proteins, albumin, globulins, and BUN were quantified. The use of supplementation did not modify these blood levels. The levels of total protein, albumin and globulins (Figure 2) were accordance with the average blood levels of the Nellore breed (Fagliari et al., 1998). We observed that globulins reached their highest level one month before calving and presented a fast decline until calving, which reflects the mobilization of immunoglobulins to produce colostrum (Weaver et al., 2000). For BUN (Figure 3), the levels observed are under the lower limit, this can be due to the limited supply of protein in the diet during pre- and/or post-calving, once BUN is synthetizing in the liver in proportional amounts, to the concentration of ammonia produced in the rumen and the blood concentration of BUN is directly correlated with the protein levels in the supplement and with the rate energy/protein in the diet (Wittwer et al., 1993).

On the other hand, the accelerated growth of the calf at the end of gestation and the milk production at the beginning of lactation, are characteristics that make of the peripartum period the time of greatest attention for the beef cow in relation to their nutritional status. An efficient way of interpreting the nutritional status of the cow is the identification of the NEB. NEFA and β HB blood levels are significant for the evaluation of energetic status in ruminants, responding rapidly to changes in food intake, thus indicating the productive capacity of a cow and thus assist in estimating the magnitude of NEB. (Peixoto and Osório, 2007; Mulliniks et al., 2013).

The results from this study indicate that, during postpartum the beef cows were susceptible to metabolic dysfunction. According to Lopes et al. (2016b) studied Nellore

cows which received a supplementation during post-calving, and observed up to postpartum mean values above 0.43 mmol/L for NEFA, in this way they suggest that supplementation is not able to increase energy intake to avoid NEB. Therefore, the mean NEFA values found in this study between the samples of days 14 and 42 post-partum (Figure 4), could be an indication of intense mobilization of body reserves. The similar behavior presented by the blood concentration of β HB (Figure 4) contributes with the acceptance of the results of this study. After the day 49 post-partum the serum concentrations of NEFA and β HB, initiated a decrease in the search for adequate levels. Overall, few studies indicate what threshold of serum NEFA would be considered where there is a high lipid mobilization in beef cows. According to Oetzel (2004), values greater than 0.40 mmol already suggests problems concerning energy balance.

An increased blood NEFA concentration directly impairs ovarian cyclicity (Kendrick et al., 1999). A thorough understanding of the many interacting factors, including genetics, nutrition, body energy reserves, health and mainly fertility is required to optimise overall cow productivity, (Diskin and Kenny, 2014). In order to understand the fertility of beef cows, the behavior of progesterone should be emphasized, since it acts as the main indicator of the return of ovarian cyclicity. Early resumption of ovarian cyclicity postpartum facilitates a greater number of estrus cycles before reproductive season which, on average, increases the likelihood of subsequent conception (Darwash et al., 1997). Absence of effect of supplementation on serum concentration of progesterone in beef cows (Table 9) may be indicative of an inability of the supplementation used to contribute to greater reproductive efficiency.

Conclusion

Our data suggest that 1 kg/d of 20% of CP supplement for Nellore cows grazing low quality tropical pasture during pre- and/or post-calving on is not enough to improve the performance, nutritional and metabolic characteristics, as well as, there is no carry-over effects of pre-calving supplementation on response post-calving.

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Figures and tables

Table 1. Chemical composition of supplement and forage

Item	Supl ^d		<i>Brachiaria decumbens</i>						
	Pre	Post	July	Aug	Sept	Oct	Nov	Pre ^e	Post ^f
DM ^a	845	854	346	606	569	443	380	604	380
OM ^b	894	894	926	931	924	937	940	920	940
CP ^b	195	209	61	52	47	52	68	51	68
EE ^b	19	25	8	7	6	9	10	9	10
NDFap ^b	185	201	641	678	695	613	607	674	607
NFC ^b	514	484	224	201	182	271	265	195	265
iNDF ^b	5,0	4,7	267	394	334	253	216	293	216
NDIN ^c	163	207	254	239	229	262	272	218	272

Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber corrected for ash and protein (NDFap) non-fiber carbohydrate (NFC) indigestible neutral detergent fiber (iNDF), insoluble neutral detergent nitrogen (NDIN).

^a/ g/kg as fed.

^b/ g/kg DM.

^c/ g/kg total nitrogen.

^d/ Supplementation during pre- and post-calving.

^e/ Means from samples obtained by hand plucking sampling for the digestibility trial during the pre-calving period.

^f/ Means from samples obtained by hand plucking sampling for the digestibility trial during the post-calving period.

Table 2. Least squares means (\pm standard error) and *P*-values for effect of supplementation on cows' intake during pre-calving

Item ^a	Treatments		<i>P</i> -value
	No supplementation	Supplementation	
	kg/d		
DM	8.33 \pm 0.211	9.25 \pm 0.204	0.089
DMF	8.33 \pm 0.211	8.34 \pm 0.204	0.995
OM	7.69 \pm 0.195	8.52 \pm 0.189	0.094
CP	0.42 \pm 0.014	0.60 \pm 0.013	0.010
NDFap	5.95 \pm 0.151	6.12 \pm 0.146	0.501
iNDF	2.44 \pm 0.060	2.49 \pm 0.061	0.643
dOM	3.12 \pm 0.291	3.93 \pm 0.288	0.186
dNDF	3.10 \pm 0.178	3.21 \pm 0.175	0.696
	g/kg body weight		
DM	16.42 \pm 0.621	17.76 \pm 0.610	0.268
DMF	16.42 \pm 0.608	16.01 \pm 0.597	0.671
OM	15.17 \pm 0.573	16.35 \pm 0.563	0.278
NDFap	11.75 \pm 0.429	11.73 \pm 0.436	0.970
iNDF	4.82 \pm 0.179	4.78 \pm 0.176	0.901

^a/Total dry matter intake (DM), dry matter of forage intake (DMF), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ash and protein (NDFap), indigestible NDF (iNDF), digested organic matter (dOM), disgestible NDF (dNDF).

Table 3. Least squares means (\pm standard error) and *P*-values for effect of supplementation on apparent digestibility and synthesis of nitrogen compounds during pre-calving

Item ^a	Treatments		<i>P</i> -value
	No supplementation	Supplementation	
OM	40.35 \pm 2.474	45.93 \pm 2.455	0.250
CP	26.26 \pm 2.189	34.46 \pm 2.153	0.116
NDFap	51.89 \pm 1.663	52.27 \pm 1.643	0.886
dOM	372.6 \pm 22.80	422.7 \pm 22.62	0.258
NMic	87.78 \pm 8.674	95.68 \pm 8.629	0.584
NMic/NI	1.26 \pm 0.072	1.00 \pm 0.071	0.130
Emic	156.8 \pm 7.256	151.6 \pm 6.456	0.643

^a/ Organic matter (OM, %), crude protein (CP, %), neutral detergent fiber corrected for ashes and protein (NDFap, %), digested organic matter (dOM, g/kg DM), ruminal synthesis of microbial nitrogen (NMic, g/d), NMic in relation to N intake (NMic/NI), efficiency for synthesis of microbial protein (Emic, g microbial CP/kg dOM intake).

Table 4. Least squares means (\pm standard error) and *P*-values for effect of supplementation on cows' performance during pre-calving

Item^a	Treatments		<i>P</i>-value
	No supplementation	Supplementation	
BW _{calving}	510 \pm 10.99	531 \pm 10.56	0.301
ADG	0.102 \pm 0.100	0.357 \pm 0.095	0.207
BCS _{calving}	5.19 \pm 0.129	5.54 \pm 0.121	0.186
EGP8 _{calving}	4.99 \pm 0.544	5.77 \pm 0.509	0.405
CBW	34.17 \pm 2.410	35.76 \pm 2.378	0.499

^a/ BW at calving (BW_{calving}, kg), Average Daily Gain (ADG, kg), Body Score Condition at calving (BSC_{calving}), Back Fat at calving (EGP8_{calving}, mm), Calf Birth Weight (CBW, kg).

Table 5. Least squares means (\pm standard error) and *P*-values for effect of supplementation on serum profile of different metabolites of cows during pre-calving

Item	Treatments		Sup	<i>P</i> -value	
	No supplementation	Supplementation		Time	Sup \times Time
Glucose, mg/dL	64.81 \pm 1.116	65.04 \pm 1.095	0.894	<0.001	0.178
Total Proteins, g/dL	7.25 \pm 0.098	6.98 \pm 0.097	0.195	<0.001	0.360
Albumin, g/dL	3.30 \pm 0.051	3.40 \pm 0.050	0.335	<0.001	0.135
Globulins, g/dL	3.94 \pm 0.110	3.61 \pm 0.107	0.169	<0.001	0.731
BUN, mg/dL ^a	8.59 \pm 0.411	9.83 \pm 0.394	0.163	<0.001	0.109
NEFA, mmol/L ^a	0.30 \pm 0.032	0.27 \pm 0.032	0.508	<0.001	0.128
β HB, mg/dL ^a	3.80 \pm 0.222	3.31 \pm 0.224	0.266	0.733	0.219

^a/ Non-esterified fatty acids (NEFA); β -hydroxybutyrate (β HB); blood urea nitrogen (BUN).

Table 6. Least squares means (\pm standard error) and *P*-values for effect of supplementation on cows' intake during post-calving

Item ^a	Treatment ^b				<i>P</i> -value		
	PrePost	Pre	Post	Control	Contrasts		
					Pre	Post	Pre \times Post
	kg/d						
DM	10.85 \pm 0.665	9.57 \pm 0.658	10.57 \pm 0.674	9.68 \pm 0.665	0.897	0.180	0.785
DMF	9.92 \pm 0.592	9.57 \pm 0.585	9.63 \pm 0.603	9.68 \pm 0.592	0.885	0.817	0.756
OM	9.93 \pm 0.565	8.71 \pm 0.558	9.67 \pm 0.575	8.81 \pm 0.565	0.889	0.139	0.776
CP	0.80 \pm 0.125	0.58 \pm 0.126	0.78 \pm 0.126	0.60 \pm 0.125	0.984	0.180	0.921
NDFap	7.01 \pm 0.273	6.59 \pm 0.265	6.82 \pm 0.283	6.66 \pm 0.273	0.842	0.346	0.671
iNDF	2.27 \pm 0.074	2.15 \pm 0.071	2.21 \pm 0.078	2.17 \pm 0.074	0.821	0.354	0.632
dOM	4.63 \pm 0.451	3.96 \pm 0.449	4.73 \pm 0.455	3.91 \pm 0.451	0.961	0.175	0.865
dNDF	3.87 \pm 0.171	3.77 \pm 0.166	3.89 \pm 0.177	3.71 \pm 0.171	0.945	0.461	0.815
	g/kg body weight						
DM	23.35 \pm 1.234	19.76 \pm 1.223	22.30 \pm 1.250	20.55 \pm 1.234	0.918	0.096	0.499
DMF	21.34 \pm 1.153	19.76 \pm 1.141	20.30 \pm 1.170	20.55 \pm 1.153	0.917	0.594	0.473
OM	20.93 \pm 0.989	17.98 \pm 0.953	20.06 \pm 0.989	18.70 \pm 0.964	0.938	0.091	0.459
NDFap	15.08 \pm 0.453	13.58 \pm 0.440	14.37 \pm 0.469	14.13 \pm 0.490	0.870	0.129	0.236
iNDF	4.88 \pm 0.119	4.43 \pm 0.113	4.65 \pm 0.124	4.61 \pm 0.119	0.847	0.110	0.159

^a/ Total dry matter intake (DM), dry matter of forage intake (DMF), organic matter (OM), crude protein (CP), neutral detergent fiber corrected for ashes and protein (NDFap), indigestible NDF (iNDF), digested organic matter (dOM), disgestible NDF (dNDF). ^b/Supplementation during pre- and post-calving (PrePost), supplementation during pre-calving (Pre), supplementation during post-calving (Post), and no supplementation during pre- or post-calving (Control).

Table 7. Least squares means (\pm standard error) and *P*-values for effect of supplementation on apparent digestibility and synthesis of nitrogen compounds during post-calving

Item ^a	Treatments ^b				<i>P</i> -value		
	PrePost	Pre	Post	Control	Contrast		
					Pre	Post	Pre \times Post
OM	46.49 \pm 2.059	45.52 \pm 2.046	48.81 \pm 2.078	44.35 \pm 2.059	0.794	0.258	0.444
CP	45.76 \pm 3.020	38.26 \pm 2.993	48.14 \pm 3.060	39.53 \pm 3.020	0.579	0.056	0.863
NDFap	56.14 \pm 0.698	57.22 \pm 0.604	57.15 \pm 0.662	55.77 \pm 0.631	0.747	0.826	0.131
dOM	425.9 \pm 17.11	414.3 \pm 16.98	446.9 \pm 17.29	403.7 \pm 17.11	0.776	0.185	0.406
NMic	98.48 \pm 14.01	85.63 \pm 14.01	93.39 \pm 14.17	83.77 \pm 14.17	0.817	0.470	0.914
NMic/NI	0.79 \pm 0.088	0.92 \pm 0.088	0.75 \pm 0.090	0.85 \pm 0.090	0.541	0.264	0.845
Emic	131.1 \pm 9.111	130.6 \pm 9.111	122.1 \pm 9.354	117.9 \pm 9.566	0.309	0.814	0.849

^a/ Organic matter (OM, %), crude protein (CP, %), neutral detergent fiber corrected for ashes and protein (NDFap, %), digested organic matter (dOM, g/kg DM), ruminal synthesis of microbial nitrogen (NMic, g/d), NMic in relation to N intake (NMic/NI, g/g ingested N), efficiency for synthesis of microbial protein (Emic, g microbial CP synthesis kg dOM intake). ^b/Supplementation during pre- and post-calving (PrePost), supplementation during pre-calving (Pre), supplementation during post-calving (Post), and no supplementation during pre- or post-calving (Control).

Table 8. Least squares means (\pm standard error) and *P*-values for effect of supplementation on cows' performance during post-calving

Item ^a	Treatments ^b				<i>P</i> -value		
	PrePost	Pre	Post	Control	Pre	Post	Pre \times Post
BW _{post_end}	464 \pm 16.35	486 \pm 15.72	478 \pm 17.09	473 \pm 16.35	0.986	0.650	0.454
ADG	-0.15 \pm 0.188	-0.01 \pm 0.178	0.08 \pm 0.185	0.04 \pm 0.181	0.593	0.978	0.521
BCS _{post_end}	5.00 \pm 0.185	5.08 \pm 0.177	4.65 \pm 0.194	4.72 \pm 0.185	0.130	0.687	0.987
EGP8 _{post_end}	3.13 \pm 0.472	4.04 \pm 0.452	3.56 \pm 0.495	2.80 \pm 0.472	0.440	0.878	0.154
BW _{calf_60d}	92.18 \pm 4.658	95.04 \pm 4.681	95.07 \pm 4.965	98.33 \pm 4.811	0.479	0.485	0.963

^a/BW at the end of the post-calving period (BW_{post_end}, kg), average daily gain (ADG, kg), body score condition at the end of the post-calving period (BCS_{post_end}), back fat at the end of the post-calving period (EGP8_{post_end}, mm), calf BW at 60 days (BW_{calf_60d}, kg). ^b/Supplementation during pre- and post-calving (PrePost), supplementation during pre-calving (Pre), supplementation during post-calving (Post), and no supplementation during pre- or post-calving (Control).

Table 9. Least squares means (\pm standard error) and *P*-values for effect of supplementation on serum profile of different metabolites of cows during post-calving

Item	Treatments ^b				<i>P</i> -value		
	PrePost	Pre	Post	Control	Sup	Time	Sup \times Time
Glucose, mg/dL	63.13 \pm 2.316	59.61 \pm 2.325	61.09 \pm 2.468	63.40 \pm 2.316	0.655	<0.001	0.143
Total Proteins, g/dL	6.85 \pm 0.101	6.68 \pm 0.096	6.76 \pm 0.106	6.85 \pm 0.096	0.610	0.002	0.411
Albumin, g/dL	3.41 \pm 0.114	3.14 \pm 0.112	3.10 \pm 0.117	3.25 \pm 0.112	0.351	<0.001	0.532
Globulins, g/dL	3.44 \pm 0.190	3.51 \pm 0.188	3.66 \pm 0.195	3.60 \pm 0.187	0.846	0.067	0.272
BUN, mg/dL ^a	9.61 \pm 1.335	9.19 \pm 1.332	9.63 \pm 1.343	9.58 \pm 1.330	0.994	<0.001	0.851
NEFA, mmol/L ^a	0.48 \pm 0.083	0.38 \pm 0.083	0.40 \pm 0.083	0.36 \pm 0.083	0.749	<0.001	0.234
β HB, mg/dL ^a	5.19 \pm 0.506	5.87 \pm 0.509	5.34 \pm 0.506	5.48 \pm 0.506	0.804	<0.001	0.376
Progesterone, (ng/mL)	3.60 \pm 0.422	2.95 \pm 0.342	3.13 \pm 0.500	3.00 \pm 0.369	0.370	<0.001	0.408

^a/ Non-esterified fatty acids (NEFA); β -hydroxybutyrate (β HB); blood urea nitrogen (BUN).

^b/ Supplementation during pre- and post-calving (PrePost), supplementation during pre-calving (Pre), supplementation during post-calving (Post), and no supplementation during pre- or post-calving (Control).

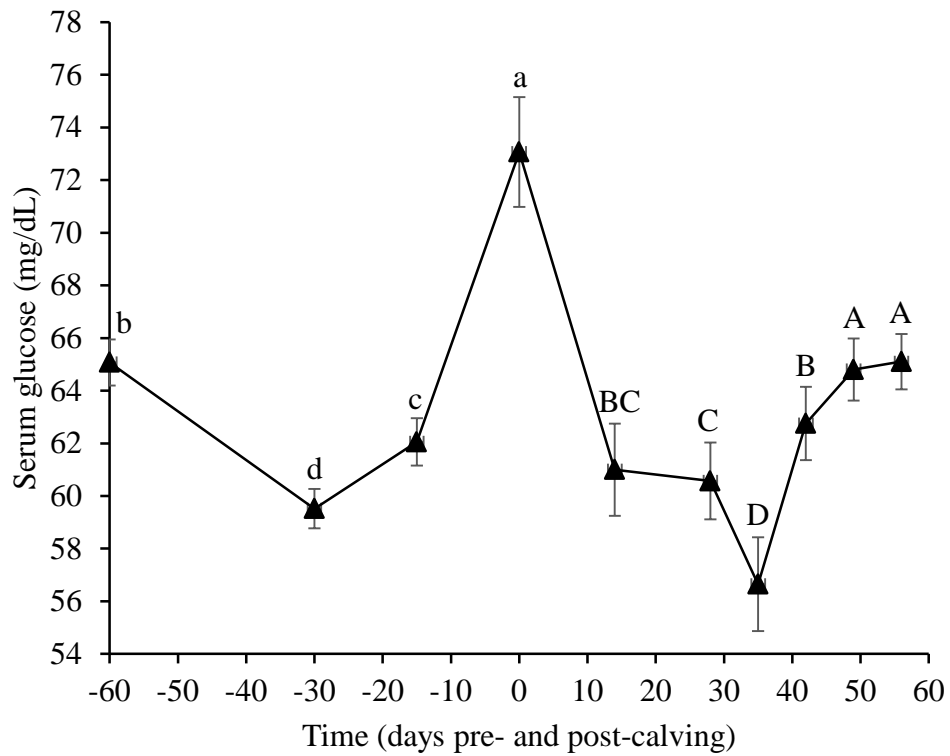


Figure 1. Serum concentration of glucose of beef cows during pre- and post-calving. Different lowercase letters declares significantly different serum concentration of glucose during pre-calving ($P < 0.10$). Different uppercase letters declares significantly different serum concentration of glucose during post-calving ($P < 0.10$) by Fisher's LSD test.

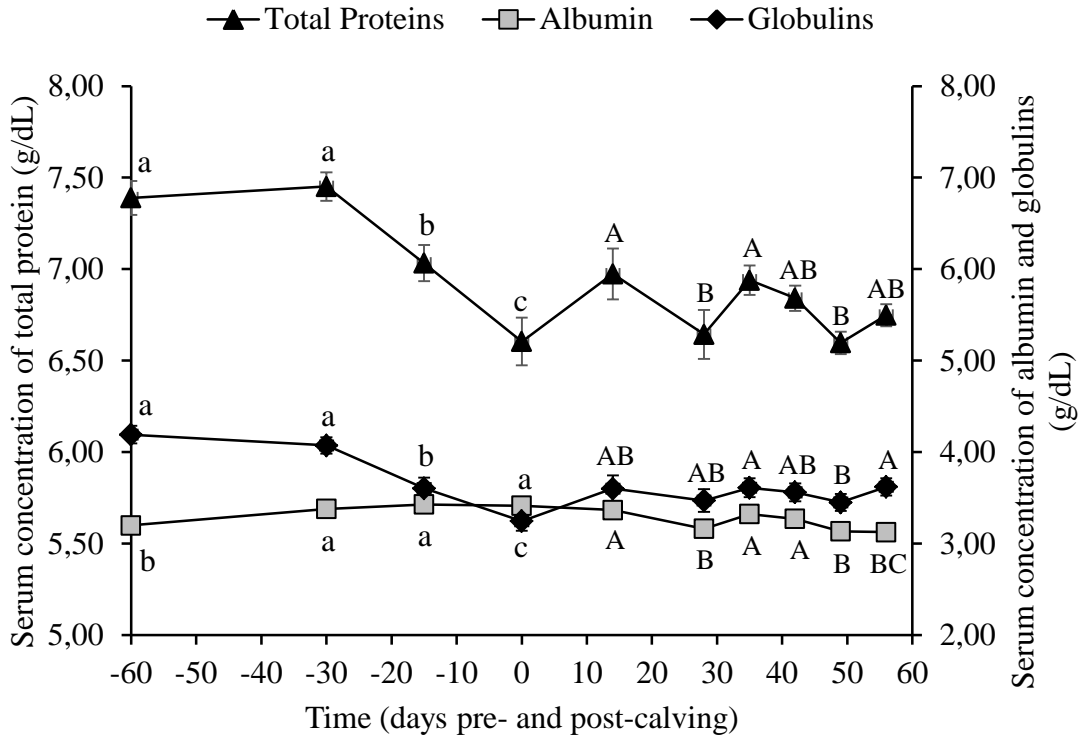


Figure 2. Serum concentration of total proteins, albumin and globulins in beef cows during pre- and post-calving. Different lowercase letters declares significantly different concentrations during pre-calving ($P < 0.10$). Different uppercase letters declares significantly different concentrations during post-calving ($P < 0.10$) by Fisher's LSD test.

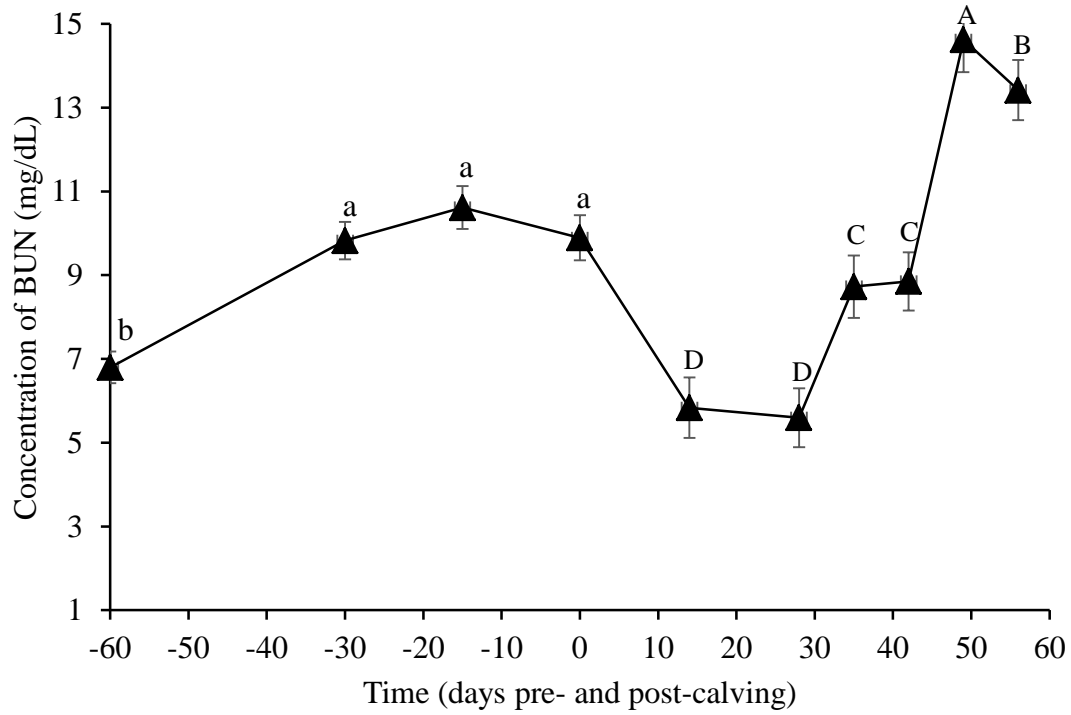


Figure 3. Serum concentration of BUN in beef cows during pre- and post-calving. Different lowercase letters declares significantly different concentrations during pre-calving ($P < 0.10$). Different uppercase letters declares significantly different concentrations during post-calving ($P < 0.10$) by Fisher's LSD test.

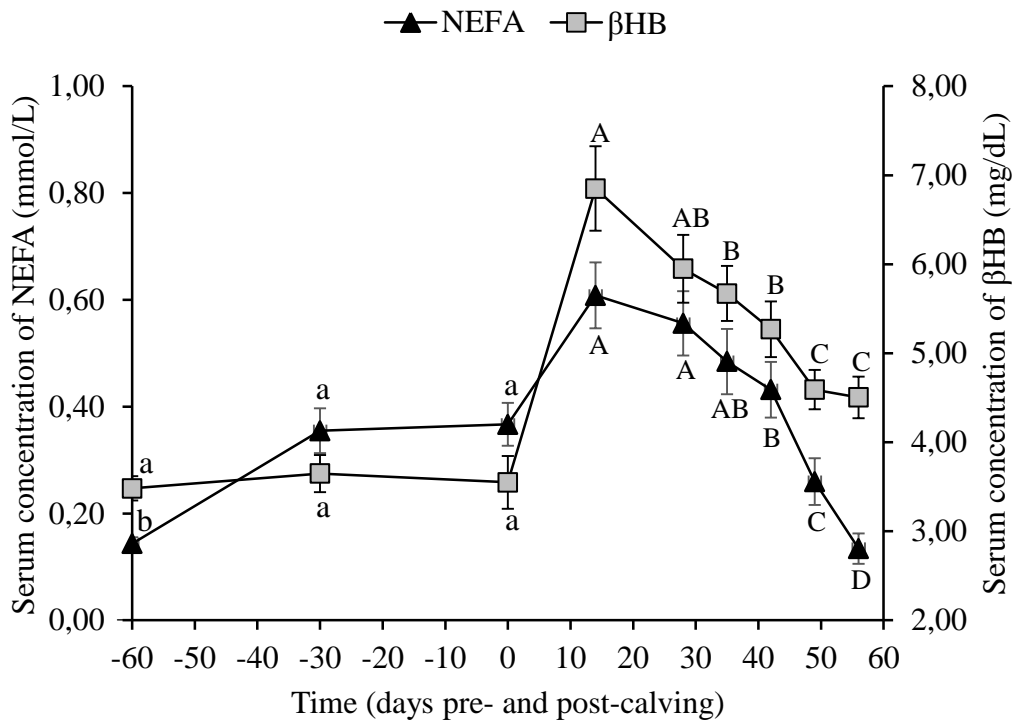


Figure 4. Serum concentration of non-esterified fatty acids (NEFA) and β -hydroxybutyrate (β HB) in beef cows during pre- and post-calving. Different lowercase letters declares significantly different concentrations during pre-calving ($P < 0.10$). Different uppercase letters declares significantly different concentrations during post-calving ($P < 0.10$) by Fisher's LSD test.

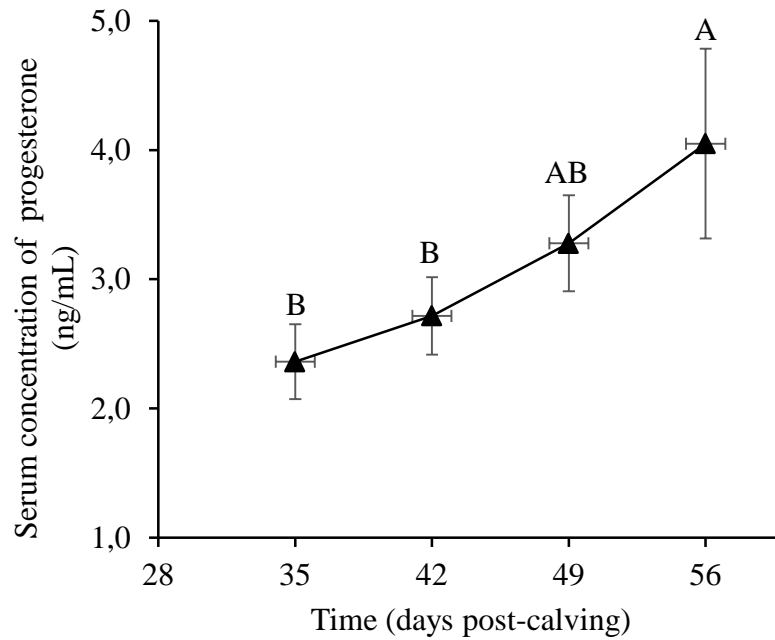


Figure 5. Serum concentration of progesterone in beef cows during post-calving. Different uppercase letters declares significantly different concentrations ($P < 0.10$) by Fisher's LSD test.