Performance, metabolic and hormonal responses of grazing Nellore cows to an energy-protein supplementation during the pre-partum phase

Matheus Fellipe de Lana Ferreira (✉ matheus.fellipe234@gmail.com)

Universidade Federal de Viçosa <https://orcid.org/0000-0002-7524-8233>

Luciana Navajas Rennó

Universidade Federal de Viçosa

Edenio Detmann

Universidade Federal de Viçosa

Mário Fonseca Paulino

Universidade Federal de Viçosa

Sebastião de Campos Valadares Filho

Universidade Federal de Viçosa

Samira Silveira Moreira

Universidade Federal de Viçosa

Hudson Caio Martins

Universidade Federal de Viçosa

Bruno Inácio Correa de Oliveira

Pontificia Universidade Catolica do Parana

Julia Avansi Marquez

Universidade de Sao Paulo

Isabela de Paula Cidrine

Universidade Federal de Viçosa

Research article

Keywords: nutrition, metabolism, parturition, Zebu

Posted Date: January 24th, 2020

DOI: <https://doi.org/10.21203/rs.2.17836/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Supplementation programs for prepartum beef cows are usually adopted because the nutritional status of the cows upon calving is a main factor impacting reproductive performance of the next production cycle. Metabolic parameters that relate nutritional status to physiological processes can be used to better understand the effects of supplementation. This study evaluated the effects of 60-d prepartum energy-protein supplementation on performance, metabolic and hormonal responses during the peripartum phase of grazing beef cows. Thirty-eight pregnant multiparous Nellore cows were assigned to a completely randomized design with two treatments: control (no supplement) and supplementation (1.5 kg of energy-protein per d) during the last 60 d of gestation. **Results:** The supplemented cows had higher ADG pre-partum ($P<0.10$), but postpartum ADG did not differ between treatments. Nonsupplemented cows' ADG did not change during these periods ($P>0.10$). Supplementation did not affect BCS and calf BW upon calving on days 45 and 90, milk yield and composition ($P>0.10$). No differences were found for forage intake and neutral detergent fiber digestibility ($P>0.10$). The intake and digestibility of CP and OM increased with supplementation ($P<0.10$). An interaction occurred ($P>0.10$) between supplementation and peripartum days for BUN, β HB, T3 and T4. Concentration of others blood parameters significantly changed ($P<0.10$) along peripartum days. There was no difference in pregnancy rates and days from calving to conception among treatments ($P>0.10$). **Conclusions:** Providing an energy and protein supplement to grazing Nellore cows over the last 60 d of gestation improved their pre-partum energy balance. However, no post-partum carryover effects were detected. **Keywords:** nutrition, metabolism, parturition, Zebu

Background

The nutritional status upon calving is the main factor influencing duration between calving and the next conception [1,2]. It affects reproductive performance and, consequently, the economic success of any beef cattle operation. Inadequate dietary energy supply during late gestation impairs reproduction, even when it is sufficient during lactation. Hence, there is clear evidence that supplementation of beef cows pre-partum is more important than postpartum [3,4].

As beef cows spend most gestation period during the dry season period (lower forage yield and quality), energy-protein supplementation can be adopted for tropical conditions to ensure grazing cattle's better performance [5,6].

It has been recently shown that a supplementation period over the last 60 d prepartum decreases the negative energy balance during the postpartum phase and reduces the number of days from calving to conception [7]. However, other experiments do not corroborate this [8,9] and show inconsistent effects of late gestation supplementation on performance and metabolic parameters.

Changes in blood hormones and metabolite concentrations during the peripartum may be interpreted as metabolic cues that relate nutrition to physiology [10], and may help to accurately indicate the effects of

supplementation on animal metabolism. The aim of this study was to evaluate the effects of a supplementation program over 60 d pre-partum on the peripartum performance, metabolic and hormonal responses of grazing Nellore cows.

Results

The average dry matter (DM) and potentially digestible DM (pdDM) availabilities during the experiment were 2.74 t/ha and 1.70 t/ha.

An interaction occurred between treatment and period (pre-partum and postpartum) for ADG ($P < 0.10$). The SS cows had higher ADG during the pre-partum ($P < 0.10$), but ADG did not differ between treatments during the postpartum period. ADG in the NS cows did not vary among periods ($P > 0.10$; Figure 1).

Supplementation did not affect calving BW, calving BCS and 45-d post-partum, CBW and calf weight at 45- and 90-d, days from parturition to conception, pregnancy rate (Table 3), milk yield and composition at 30- and 45-d lactation ($P > 0.10$) (Table 4). Total DM, organic matter (OM), crude protein (CP) and digestible OM intake were higher ($P < 0.10$) for the supplemented cows, but forage (DMF), apNDF and iNDF intake were not affected by treatments ($P > 0.10$; Table 5). However, when expressed in g/kg BW, the above-cited intakes were not influenced by supplement supply ($P > 0.10$).

Supplementation during the pre-partum improved digestibility of OM and CP ($P < 0.10$). It had no effect on apNDF digestibility or NMic, but affected microbial protein synthesis efficiency (Emic, g CP/kg dOM), which was higher for the NS animals ($P > 0.10$; Table 6).

No interaction was observed between supplement and peripartum days for blood glucose concentration, triglycerides, total cholesterol, HDL, LDL and VLDL ($P > 0.10$; Table 7) and supplementation did not affect these variables ($P > 0.10$). However, the concentration of these variables changed significantly during peripartum days ($P < 0.10$).

For glucose (Figure 2), higher serum concentrations were observed upon calving (day 0 - 80,37 mg/dL), before lowering at 15-d and then stabilizing at the baseline ($P < 0.10$). Lower serum concentrations were observed for total cholesterol ($P < 0.10$; Figure 4) and LDL had upon calving, which then increased from calving to day 30. Higher HDL concentrations were recorded on day 45. Conversely, triglycerides and VLDL serum levels were higher on -30-d than for calving and post-partum (Figures 3 and 5).

Supplementation did not affect the serum concentration of total proteins, albumin, globulins and creatinine during both pre- and post-partum, and only were different during peripartum days ($P > 0.10$; Table 7).

For total proteins serum concentrations were similar on days 30 and 45 ($P > 0.10$; Figure 6a), and higher than on days -30, 0 and 15 ($P < 0.10$). Similar serum concentrations were observed for serum albumin (Figure 6b) on day -30 and upon calving ($P > 0.10$), but were higher than on days 15, 30 and 45 ($P < 0.10$). Higher serum concentrations were found for globulins (Figure 6c) on day 45 compared to the rest of the

period, which reflects the behavior of total proteins. Creatinine lowered throughout the peripartum, with the lowest values noted on days 30 and 45 postpartum ($P < 0.10$; Figure 7).

An interaction with the BUN concentrations between supplement and peripartum days occurred (Table 7), where concentrations were higher for the cows supplemented on day -30 and upon calving ($P < 0.10$), and lower on 45 d post-partum ($P < 0.10$; Figure 8).

Serum NEFA concentrations were not affected by supplementation ($P > 0.10$; Table 7). The NEFA concentration changed along peripartum days, and its concentrations on day -30 were lower than on the calving day and stabilized after 30 d (Figure 9). However, an interaction was observed between supplementation and peripartum days for β HB ($P < 0.10$; Table 7), with the lowest concentrations on -30-d for supplemented cows (Figure 10).

No effect of supplementation was found on insulin and IGF-1 levels, and it only changed on peripartum days ($P > 0.10$; Table 7) when both peaked upon calving (Figure 11). An interaction occurred for both T3 and T4 between supplementation and peripartum days ($P < 0.10$; Table 7) when concentrations were higher on day -30 for the supplemented cows (Figure 12). Supplementation did not affect progesterone serum levels (Table 7), which were higher on day 45 than on day 30 (1.60 and 0.30 ng/mL).

Discussion

Grazing cattle are usually supplemented with additional feed to increase or maintain their BCS, mainly during the dry season, or when the quality of the pasture is insufficient to meet their nutrient requirements [5,6]. Feed supplementation of grazing cattle can lead to changes in ruminal fermentation. For example, the use of energy-protein supplements increases the degradation rate of insoluble fibrous compounds by improving the energy intake extracted from forage, which thus guarantees a rise in total DM intake due to a higher passage rate [25].

Data in the literature suggest that CP levels in diet of around 100 g/kg of DM improve fiber degradation [6], and CP quantity of around 145 g/kg DM is able to improve pasture voluntary intake [26]. In this experiment, total DM intake increased basically due to the supplement added to intake. However, supplementation did not increase pasture intake and apNDF digestibility. Basically, the quantity of CP herein used in the supplement and pasture (around 81 g/kg DM) was not enough to have a positive effect on forage voluntary intake and fiber degradation, which remained below the values suggested by the above-cited authors.

The supplemented animals displayed higher CP intake and OM and CP digestibly. This was expected because supplementation provides more dietary protein intake, apart from other high digestible nutrients. As the NS had lower protein intake and OM digestibility, nitrogen utilization efficiency was apparently enhanced, which led to higher Emic.

In the last 60 gestation days, cows display higher nutritional requirements [11] and, according to Silva et al. [7], supplementation administered in adequate amounts during this period can have beneficial effects on cow's energy and protein metabolism, along with increased body reserves. Thus, it would seem to metabolically prepare cows for the postpartum period when supplements are no longer provided. Nevertheless, in the present study, and unlike Silva et al. [7] found, pre-partum supplementation had no effect on reducing the magnitude of BW lost during the post-partum as variation in ADG was noted in the SS animals during the experimental period, which was negative during the post-partum (Figure 1). These findings agree with Cardenas [8] and Sotelo et al. [9].

No difference in postpartum anestrus length was observed and supplementation had no effect on progesterone levels (Table 7). This indicates that supplementation was unable to contribute to greater reproductive efficiency. As expected, the above-cited results consequently reflect post-partum performance, with no difference found in pregnancy rate and days from calving to conception. These results might be explained by the fact that most of the cows presented appropriate BCS for reproduction at the beginning of the experiment (5 to 6.0 on a scale from 1 to 9) [28,29].

According to several works, BCS is a determining factor for cows to return to early estrus with improved conception rates [30, 3]. Furthermore, for cows with adequate BCS, there is evidence that body reserves can be used during late gestation without compromising the subsequent reproductive function [4]. This questions the need for supplementing cows with adequate BCS at the end of gestation. In other words, if grazing cows are appropriately conditioned (i.e. BCS 5 to 6) towards the end of gestation and do not mobilize body reserves extensively before calving; supplementation of extra feed will not lead to improvements of the reproductive performance during the subsequent reproductive cycle.

Although pasture had low quality (less than 7 g/kg DM of CP [25]) its availability to animals sufficed and allowed selective grazing, which led NS animals to maintain ADG throughout the experimental period (Figure 1). Therefore, no differences were found in either calves' BW or milk production. It is well-known that adequate BCS in females throughout pregnancy does not influence the BW of the progeny at birth [30,31].

In fact, these affirmations are also supported by the results of hormones and metabolites, which mainly changed only during peripartum days.

The plasma glucose concentration declined regardless of the treatment at the end of gestation, which can be explained by the higher fetal demand during this period [11]. Upon calving, cows are stressed and, therefore, epinephrine acts by stimulating glycogen catabolism [32] to minimize stress during calving. Glucocorticoids act by promoting gluconeogenesis in the liver, and decrease glucose uptake and utilization in muscle and adipose tissue [33]. Hence glucose concentrations were higher during this period. The lower glucose concentrations after calving were probably caused by not only reduced DM intake, but also by higher energy demand for milk [34]. On days 30 and 45, serum glucose levels were restored and remained at basal levels.

Cholesterol levels progressively increased on all post-partum days regardless of the supplementation period. This agrees with Ruas et al. [35] and Godoy et al. [36] for postpartum blood cholesterol in lactating beef cows. Something similar occurs with HDL concentrations, which also increased after calving. In ruminants, lactogenesis increases plasma HDL concentrations, which is possibly due to an increase in either HDL synthesis or catabolism of VLDL by mammary tissue [37]. This would explain the low triglycerides and VLDL concentrations upon post-partum, and suggest its utilization as energy demand for lactation as they are important sources of fatty acids for milk fat synthesis [38]. Increased cholesterol during the postpartum could be related to precursors being needed for the synthesis of steroidal hormones [39]. While reproductive activity is re-established, avascularized granulosa cells are restricted to cholesterol uptake from HDL [40].

Data in the literature reveal that restricting pregnant cows' intake during the late gestation period results in weight loss, BCS loss and high serum concentrations of NEFA and β HB, which lead to long periods of negative energy balance in both dairy [41,42] and beef cows [2].

In this study, NEFA concentrations were not affected by supplementation and these levels at calving indicate a higher adipose tissue rate of lipolysis [43,44]. The NEFA post-partum concentrations were lower compared to parturition, and remained at basal concentrations throughout the experimental period, which suggests the recovery of animals' nutritional status. The same occurred with β HB levels upon post-partum, which differed between treatments for the pre-partum with higher concentrations for the NS animals.

Despite the difference between treatments in β HB concentrations on day -30, these levels do not indicate intense body reserve mobilization for the NS animals, and even ADG presented differences among treatments upon the pre-partum. It is important to emphasize that most studies into energy deficit in ruminants have been done with dairy cows, and despite lack of information on beef cows serum β HB levels, it may be understood that those levels herein do not suggest severe energy deficit, rather cows had differing nutrient balances during the pre-partum.

Nevertheless, β HB levels had not influence days from calving to conception, unlike Mulliniks et al. [2] who found that low β HB concentrations were associated with an earlier conception date in beef cows. The contrasting experimental results may be explained by the fact that the β HB values that impaired reproduction cited by the above authors were higher at -30-d (0.71 mmol/L) than those of our experiment (0.48 mmol/L). Under these conditions, the mobilization of body reserves does not lead to loss of performance and reproduction.

Albumin concentrations significantly lowered after calving, which could be related to amino acids demand for milk production [45]. Although the lowest value appeared on day 45, it still fell within the reference values (3.03-3.55 g/dL) [46]. On pre-calving day 30 and upon calving, globulin lowered in relation to the rest of the period, justified by the transfer of immunity to colostrum production [47], as reflected in the behavior of the total proteins concentrations.

Unlike the other protein status indicators, BUN levels were higher for the supplemented animals only during the pre-partum period. This is basically due to the effect of supplementation, which raised ammonia in the rumen. As urea is considered a short-time protein indicator, higher BUN levels were expected during the supplement period. Urea is synthesized in the liver in proportional amounts to the concentration of ammonia produced in the rumen, and its concentration is related directly to dietary protein levels [48].

The creatinine blood concentration is an index of muscle mass, insofar that creatinine excretion is proportional to lean body mass and is, therefore, proportional to an animal's BW [49]. As expected, creatinine concentrations lowered linearly throughout the peripartum due to weight loss, with the lowest values on post-partum days 30 and 45, but still within the reference values (1-2 mg/dL) [46].

Nutritional status also regulates the production of IGF-I and insulin, which are sensitive to nutritional plan [50] and are extremely helpful parameters. Feed-restricted animals display lower serum glucose and, consequently, less insulin, which reduces the somatotropin receptors in the liver, the main mediator of IGF-I production. Thus, animals in the catabolic state have lower plasma IGF-I concentrations [51-53]. 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 to the direction of changes in BW.

During the experiment, insulin and IGF-1 levels behaved similarly and were not influenced by supplementation. Under similar conditions, Silva et al. [7] reported no difference in insulin levels of cows supplemented during the pre-partum. The higher values for both hormones were observed on the calving day, possibly due to blood glucose increasing and then lowering during early lactation as part of the homeorhetic changes to support galactopiesis [42]. IGF-1 concentrations were restored after 30-d post-partum, as were insulin levels on day 45, which thus stimulated steroidogenesis [54] and led to higher progesterone levels by day 45.

Food-restricted ruminants adapt to lower maintenance requirements by means of a slowing down the basal metabolism rate [55] due to lowering circulating levels of thyroid hormones. During this experiment, total T3 and T4 reduced during the pre-partum for the NS animals, which could be explained by a lowering metabolic rate compared to SS. Several works have also reported that cows during the post-partum with a negative energy balance respond to lower total T3 and T4 concentrations due to both the energy deficiency state and the huge demand of these hormones by mammary glands [56]. Conversely from parturition to 45-d, total T3 and T4 levels behave distinctly. Coggins and Field [57] demonstrated that, compared to T3, T4 serum concentrations were a more sensitive indicator of energy balance in lactating beef cows. This supports our observation herein of lower T4 levels on post-partum day 15, unlike T3, which did not vary much during the post-partum.

Conclusions

Providing an energy and protein supplement to grazing Nellore cows over the last 60 d of gestation improved their pre-partum energy balance. However, no post-partum carryover effects were detected.

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). Animals used in this study were provided by the Beef Cattle Farm of Animal Science Department at the University Federal de Viçosa, Viçosa-MG, Brazil, where the study was carried out, from July to December 2017.

Thirty-eight pregnant multiparous Nellore cows, with an average body weight (BW) of 515 ± 11 kg, body condition scores (BCS) of 5.5 ± 0.25 and 230 ± 10 gestation days were used. Animals were randomly divided into eight paddocks that covered seven hectares evenly covered with *Urochloa decumbens* grass on average, with free access to water and feeders. 19 cows per treatment, six paddocks with five cows each and two with four. Feeders were equipped with creep-feeding and sheltered, with space of 0.3 m per cow.

The experimental design was completely randomized, with two treatments as following: NS-control; SS-cows supplemented for the 60 pre-partum days (gestation period from 230 to 290-d). The NS cows, received only a mineral mixture (MM) as loose mesh, *ad libitum*, during gestation. SS cows were grouped with 90 kg of supplement during the pre-partum period (1.5 kg/d), accompanied by MM offered *ad libitum* supplied separately in additional feeders. The compositions of supplement, MM and pasture are shown in Tables 1 and 2.

The supplement was a loose mesh formulated to contain 30% crude protein (CP) as fed to meet around 40% of CP maintenance requirements, according to BR-Corte [11]. Supplement was always provided at 11:00h to minimize any interference of animal grazing behavior [12]. After calving, cows remained in the same paddocks, but received only MM *ad libitum* until 45 lactation days.

Experimental procedures and sampling

Cows were weighed on two consecutive days at the beginning of the experiment (60-d pre-partum), and 7-d before the expected calving day to quantify the average daily gain pre-calving (ADG_{pre}). Cows were weighed after calving and at the end of the experiment period also on two consecutive days (45-d) to quantify the average daily gain post-calving (ADG_{post}). Calves remained with dams during the experiment and were weighed immediately after birth, and also at 45 and 90-d. Body condition scores (BCS) were also recorded on a scale from 1 to 9, as recommended by NRC [13], by three experienced persons at the beginning of the experiment, upon calving and 45-d post-partum.

During the breeding season, starting on December 12, cows were synchronized, and fixed time artificial insemination (FTAI) was performed on December 23. Pregnancy diagnosis was made via transrectal 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-d, grass samples were collected by hand-plucked sampling to evaluate the forage selected by animals. Samples were collected by cutting at the ground level from five delimited areas (0.5 x 0.5 m), selected randomly in each paddock to quantify DM and DMpd. In these circumstances, all the samples were weighed, oven-dried (55°C) and then ground to pass through 1- and 2-mm screens in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA).

Intake and digestibility assay

To evaluate intake and digestibility, a trial was run for 9-d on day 45 before the estimated parturition date (around 245-d of gestation). Titanium dioxide (TiO₂) was used to estimate the fecal excretion of animals, which was wrapped in paper cartridges (20 g per animal/day) and inserted with a metal probe via the esophagus at 12:00h [14]. The first 5 trial days were used to adapt animals to TiO₂. Fecal samples were collected immediately after defecation or directly from the rectum on the last 4 days (one sample/day) at 18:00h, 14:00h, 10:00h and 06:00h. Feces samples were oven-dried (55°C) and ground to pass through 1- and 2-mm screens in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, USA). Then 25 g from all 4 days were pooled.

Indigestible neutral detergent fiber (iNDF) was used to estimate pasture dry matter intake (DMI) [15]. It was assumed that supplement consumption equaled the amount offered per animal/day.

On trial day 5, forage was collected by hand-plucked sampling at each paddock separately. These samples were used to estimate voluntary dry matter intake and forage digestibility.

On the trial last day, spot urine samples (5 mL) were collected 4 h before and after administering the supplement. Then a 10-mL compound was prepared with 5 mL of the urine collected in the morning and afternoon. Urine samples were diluted in 40 mL of H₂SO₄ (0.036 N) and then frozen (-20°C).

Milk sampling

On 30- and 45-d post-calving, milking was performed to estimate milk production. In order to empty udders, calves were separated from their mothers from 15:00h to 17:45h, when they were reunited to dams and allowed to suckle. At 18:00h, calves were once again separated from dams until the next morning. At 06:00h on the next day, cows were milked immediately after an injection of 20 UI of oxytocin (10 UI/mL; Ocitovet®, Brazil) in the mammary vein and the produced milk was weighed. The exact time when each cow was milked was recorded. Calves were kept away from their mothers until the next

milking at 06:00 to obtain a 24-hour milk production. Next 30 mL of milk were separated from each cow to evaluate milk composition. Total production was corrected to 4% fat, according to NRC [16].

Blood sampling

By taking calving day as day 0, blood samples were collected before feeding on days -30, 0, 15, 30, 45. Blood samples were collected by jugular vein puncture, using vacuum tubes with a clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) to quantify: blood nitrogen urea, total protein, albumin, triglycerides, total cholesterol, high density lipoprotein (HDL), nonesterified fatty acid (NEFA), beta-hydroxybutyrate (β HB), insulin, insulin-like growth factor (IGF-1), total triiodothyronine (T3), total thyroxine (T4), progesterone (P4) contents (only on 30 and 45-d). A tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantify the plasma glucose concentration. After collection, samples were centrifuged at $3600 \times g$ for 20 min. Serum and plasma were immediately frozen at -20°C until analyzed.

Laboratory analyses

The forage, feces and supplement samples were analyzed following the procedures described by Brazilian National Institute of Science and Technology in Animal Science (INCT-CA) [17] 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 (aNDF; index INCT-CA method F-002/1). Indigestible neutral detergent fiber (iNDF) [18] was processed at 2 mm and quantified by *in situ* incubation procedures with nonwoven textile bags (100 g/m^2) for 288 h. The fecal samples were evaluated for titanium contents using acid digestion process with concentrated sulfuric acid followed by the addition of 30% hydrogen peroxide solution and further quantification by spectrophotometry (INCT-CA method M-007/1).

With the blood samples, Bioclin® kits (Belo Horizonte, Brazil) was employed to quantify urea (K056), total protein (K031), albumin (K031), triglycerides (K117), total cholesterol (K083), HDL (K071) and glucose (K082). NEFA and β HB were analyzed using Randox® kits (FA115 and RB1007, Antrim, UK). Uric acid, creatinine and urea in urine were analyzed with kits Bioclin® (K0139, K067 and K056, Belo Horizonte, Brazil). All the above-mentioned analyses were determined by an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China). Allantoin in urine was analyzed by the colorimetric method [19].

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 with kits DiaSorin® (California, USA) in an automated chemiluminescence analyzer (Liaison®, Saluggia, Italy). Milk was analyzed for protein, fat, lactose and total solids content using infrared spectroscopy (Foss MilkoScan FT120, São Paulo, Brazil).

(See Calculations and Statistical Analysis in the Supplementary Files)

List Of Abbreviations

ADGpre: average daily gain pre-calving; ADGpost: average daily gain post-calving; apNDF: Neutral detergent fiber corrected for ash and protein residue; BCS: Body condition score; β HB: beta-hydroxybutyrate; BUN: Blood urea N; BW: Body weight; CP: Crude protein; DM: Dry matter; FTAI: Fixed time artificial insemination; HDL: high density lipoprotein; IGF-1: insulin-like growth factor; iNDF: Indigestible neutral detergent fiber; MM: mineral mixture; NDF: Neutral detergent fiber; NEFA: nonesterified fatty acid; NS: Cows not supplemented during gestation; OM: Organic matter; P4: progesterone; pdDM: potentially digestible dry matter; SS: Sows supplemented for the 60 pre-partum days; T3: total triiodothyronine; T4: total thyroxine.

Declarations

Ethics approval and consent to participate

This manuscript does not involve any human subject, or human data. 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).

Consent for publication

This manuscript does not contain any individual personal data.

Availability of data and materials

The data generated during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Funding

This research was supported by funding from CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico, CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, FAPEMIG – Fundação de Amparo à Pesquisa de MG and INCT-CA - Instituto Nacional de Ciência e Tecnologia de Ciência Animal.

Authors' contributions

MFLF, conceived the study, carried out the experimental trial, performed the statistical analysis and chemical analyses, and wrote the manuscript. LNR, contributed to draft the manuscript and coordinate the research group. ED and MFP, contributed to the designing the experiment, statistical analysis and to draft the manuscript. SCVF contributed to data interpretation and to draft the manuscript. SSM, HCM, BICO, JAM, IPC carried out the experimental trial, performed the chemical analysis and contributed to draft the manuscript. All authors read and approved the final manuscript

Acknowledgements

The authors thank to CNPq, Capes, INCT-CA and FAPEMIG for financial support.

References

1. Baruselli PS, Reis EL, Marques, MO, Nasser LF, Bó GA. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Anim Rep Sci*. 2004;120:479-480.
2. Mulliniks JT, Kemp ME, Endecott RL, Cox SH, Roberts AJ, Waterman RC et al. Does β -hydroxybutyrate concentration influence conception date in young postpartum range beef cows. *J Anim Sci*. 2013;91-2902-2909.
3. Hess BW, Lake SL, Scholljegerdes EJ. Nutritional controls of beef cow reproduction. *J Anim Sci*. 2005;83:90-106.
4. Diskin MG and Kenny DA. Managing the reproductive performance of beef cows, *Theriogenology*. 2016;86:379-387.
5. Paulino MF, Detmann E, Valadares Filho SC, Silva AG, Cabral CHA, Valente EEL et al. Bovinocultura programada. In *Anais do VII Simpósio De Produção de Gado de Corte*. Viçosa;2010:267-297.
6. Detmann E, Valadares Filho SC, Paulino MF, Huhtanen, P. Nutritional aspects applied to grazing cattle in tropics: a review based on Brazilian results. *Semina*, 2014a;35:2829-2854.
7. Silva AG, Paulino MF, Detmann E, Fernandes HJ, Amorin LS, Ortega REM et al., Energetic-protein supplementation in the last 60 days of gestation improves performance of beef cows grazing tropical pastures. *J Anim Sci Biotech*. 2017;8:78-87.
8. Cardenas JEG. Nutritional and metabolic evaluation of Nelore cows supplemented or not during the peripartum. PhD Thesis – Federal University of Viçosa, Animal Science Department, 2017.
9. Sotelo D, Paulino MF, Rennó LN, Detmann E, Ortega RM, Marquez DC et al. Performance and metabolic status of grazing beef heifers receiving increasing protein supplementation pre- and postpartum. *Anim Prod Sci*.
10. Payne JM, Payne S. The metabolic profile test. Oxford, Oxford University, in press, 1987.
11. 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.

12. Adams DC. Effect of time of supplementation on performance, forage intake and grazing behavior of yearling beef steers grazing Russian wild ryegrass in the fall. *J Anim Sci.* 1985;61:1037-1042.
13. National Research Council – NRC. Nutrient requirements of beef cattle. 7th ed. Washington: Academic Press; 2000.
14. Titgemeyer EC, Armendariz CK, Bindel DJ, Greenwood RH, Loest C A. Evaluation of titanium dioxide as a digestibility marker for cattle, *J Anim Sci.* 2001;79:1059-1063.
15. Detmann E, Paulino MF, Zervoudakis JT, Valadares filho SC, Euclydes RF, Lana RP et al. Chromium and internal markers in intake determination by crossbred steers, supplemented at pasture. *Rev Bras Zootec.* 2001;30:1600-1609.
16. National Research Council – NRC. Nutrient requirements of beef cattle. 7th ed. Washington: Academic Press; 2001.
17. Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, et al. Métodos para análise de alimentos – INCT – Ciência Animal. Visconde do Rio Branco. Brazil: Suprema; 2012. 214p.
18. Valente TNP, Detmann, E, Queiroz AC, Valaderes filho SC, Gomes DI, Figueiras JF. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. *Bras. Zootec.* 2011;40:2565-2573.
19. Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle basid on urinary excretion of purine derivatives-an overview of the technical details. Ocasional publication. Ed. Rowett Research Institute: Buchsburnd Aberdeen, UK; 1992. 21p
20. Paulino MF, Detmann E, Valente EEL, Barros LV. Nutrição de bovinos em pastejo. *In Simpósio Sobre Manejo Estratégico da Pastagem.* Viçosa; 2008:131-169.
21. Costa e Silva LF, Valadares Filho SC, Chizzotti ML, Rotta PP, Prados LF, Valadares RFD, et al. Creatinine excretion and relationship with body weight of Nellore cattle. *Rev. Bras. Zootec.* 2012;41:807-810
22. Gionbelli MP, Valadares Filho SC, Detmann E. Adjusting cattle body weight to physiological and feeding conditions. *In BR-CORTE 3.0 - Nutrient requirements of zebu and crossbred cattle.* 3rd Ed. Viçosa: Universidade Federal de Viçosa; 2016
23. Barbosa AM, Valadares RFD, Valadares Filho SC, Pina DS, Detmann E, Leão MI. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle. *J Anim Sci,* 2011;89:510-519.
24. Friedewald WT, Levy RI, Fredrickson, DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry.* 1972;18:499–502.
25. Lazzarini I, Detmann E, Sampaio CB, Paulino MF, Valadares Filho SC, Souza MA, et al. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Rev Bras Zootec.* 2009;38:2021–2030.

26. Detmann E, Valente EEL, Batista ED, Huhtanen P. An evaluation of performance and efficiency of nitrogen utilization in cattle feed tropical grass pastures with supplementation. *Liv Sci*. 2014b;162:141-153.
27. Lowman BG, Scott NA and Somerville SH. *Condition scoring of cattle*. Edinburgh: Edinburgh School of Agriculture. 1976.
28. Randel DR. Nutrition and postpartum rebreeding in cattle. *J Anim Sci*. 1990;68:853-862.
29. Bishop DK, Wettemann RP and Spicer LJ. Body energy reserves influence the onset of luteal activity after early weaning of beef cows. *J Anim Sci*. 1994;72:2703-2708.
30. Marques RS, Cooke RF, Rodrigues MC, Moriel P, Bohnert DW. Impacts of cow body condition score during gestation on weaning performance of the offspring. *Liv Sci*. 2016;191:174–178.
31. Marquez DC, Paulino MF, Rennó LN, Villadiego FC, Ortega RM, Moreno DS et al. Supplementation of grazing beef cows during gestation as a strategy to improve skeletal muscle development of the offspring. *Animal*. 2017;1-9.
32. Kolnes AJ, Birk JB, Eilertsen E, Stuenæs JT, Wojtaszewski JF, Jensen J. Epinephrine-stimulated glycogen breakdown activates glycogen synthase and increases insulin-stimulated glucose uptake in epitrochlearis muscles. *Anim J Physiol Endocrinol Metab*. 2015;308:231-240.
33. Foster LA. Clinical ketosis. *Vet Clin North America-Food Anim Prac*. 1998; 4:253–267.
34. Larson, BL. *Lactation*. Iowa State University Press, Ames; 1985.
35. Ruas JRM, Torres, CAA, Valadares Filho SB, Pereira JC, Lazaro EB, Neto, AM. Efeito da suplementação proteica a pasto sobre consumo de forragens, ganho de peso e condição corporal, em vacas Nelore. *Bras. Zootec*. 2000; 29:930-934.
36. Godoy MM, Alves JB, Monteiro ALG, Valério Filho WV. Reproductive and Metabolic Parameters of Guzera Cows Supplemented in Pre and Postpartum. *Rev Bras Zootec*. 2004; 1:103-111.
37. Puppione DL. Implications of unique features of blood lipid transport in the lactating cow. *J Dairy Sci*. 1978; 61:651.
38. Aeberhard K, Bruckmaier R M, Blum J W. Metabolic, enzymatic and endocrine status in high yielding dairy cows – Part 2. *Journal of Veterinary Medicine*. 2001;8:111-127.
39. Holtenius K, Agenas S, Delavaud C, Chilliard Y. Effect of feeding intensity during the dry period. 2. metabolic and hormonal responses. *J Dairy Sci*. 2003;86:883-891.
40. Shalgi R, Kraicer P, Rimon A, Pinto M, Soferman N. Protein of human follicular fluid: the blood follicle barrier. *Steril*. 1973;24:429.
41. Barber MC, Clegg RA, Travers MT, Vernon RG. Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta*. 1997;1347:101–126.
42. Bauman DE. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. In *Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction. Proceedings of the 9th International Symposium on Ruminant Physiology*. Edited by Cronje PB. New York: CABI Publishing. 2000:311–328.

43. Astessiano A L, Pérez-Clariget R, Espasandí AC, López-Mazz C, Soca P, Carriquiry, M. Metabolic, productive and reproductive responses to postpartum short-term supplementation in primiparous beef cows. 2013;42:246-253.
44. Lopes SA, Paulino MF, Detmann E, Rennó LN, Valente EELV, Cabral CHA et al. Evaluation of grazing beef cows receiving supplements with different protein contents. *Semina: Ciên Agr*, 2016;37:3361-3372.
45. Contreras P. Indicadores do metabolismo proteico utilizados nos perfis metabólicos de rebanhos. *In Perfil Metabólico em ruminantes: seu uso em nutrição e doenças nutricionais*. Volume 1. Edited by González FHD, Barcellos JO, Ospina H, Ribeiro LAO. Porto Alegre, Brasil:Gráfica da Universidade Federal do Rio Grande do Sul; 2000:21-30.
46. Kaneko, JJ, Harvey, JW, Bruss ML. *Clinical biochemistry of domestic animals*. San Diego, Academy Press.
47. Weaver DM, Tyler JW, Vanmetre DC. et al. Passive transfer of colostral immunoglobulins in calves. *J Vet Inter Med*. 2000;14:569-577.
48. Owens FN and Bergen WG. Nitrogen metabolism in ruminant animals, historical perspective, current understanding and future implications. *J Anim Sci*. 1983; 57:(Suppl. 1).
49. Lofgreen GP, Garret, WN. Creatinine excretion and specific gravity as related to the composition of the 9, 10, 11th rib cut of Hereford steers. *J Anim Sci* 1954;13:496–500.
50. Clemmons DR, Dehoff, M, McCusker, R. et. al. The role of insulin-like growth factor in the regulation of growth. *J Anim Sci*, 1987;(Suppl 2):165-170.
51. McGuire MA, Vicini, JL, Bauman DE and Veenhuizen JJ. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *J Anim Sci*. 1992;70:2901– 2910.
52. Thissen JP, Ketelslegers JM, and Underwood LE. Nutritional regulation of the insulin-like growth factors. *Rev*. 1994;15:80-101.
53. Yambayamba ES, Price MA, Foxcroft GR. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers, *J Anim Sci*. 1996;74:57-69.
54. Wettemann RP, Bossis I. Energy intake regulates ovarian function in beef cattle. *J Anim Sci*. 2000;77(Suppl 1): E1–E10.
55. Chilliard Y, Bocquier F, Doreall M. Digestive and metabolic adaptation of ruminants to undernutrition and consequences on reproduction. *Rep Nutr Dev*, 1998; 2:131-152.
56. Ronge H, BLUM J, Clement C, Fans F. Somatomedin C in dairy cows related to energy and protein supply and to milk production. *Prod*. 1988;47:165.
57. Coggins CR and Held AC. 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 Blood Profiles in Animal Production*. Edited by Lister D. *Brit Soc Anim Prod*. 1977;1:41-47.

Tables

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

Item	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

□ 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□	651.3	762.3	505.8	236.7
OM□	931.8	937.6	934.1	910.4
CP□	48.8	52.3	58.3	82.1
apNDF□	749.3	770.1	731.2	592.5
iNDF□	338.7	347.6	362.1	177.8
NDIN□	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).

□/ g/kg of natural matter

□/ g/kg DM

□/ g/kg total nitrogen

⁴/ intake and digestibility assay

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

Item	Treatment		SEM	P-value
	NS	SS		
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

▯BW- body weight; BCS – Body Condition Score; CBW - Calf Birth Weight; CBW45- Calf Body Weight at 45-d; CBW90- Calf Body Weight at 90-d

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

Item	Treatment		SEM	P-value
	NS	SS		
Milk 30 (kg)	7.69	7.84	0.432	0.817
FCM ^a 30 (kg)	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 (kg)	7.74	8.15	0.424	0.525
FCM ^a 45 (kg)	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
------------------	-------	-------	-------	-------

^aFMC=4% fat-corrected milk yield (30 and 45-d)

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

Item	Treatments		SEM	P-value
	NS	SS		
	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), digested NDF (dNDF).

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

Item	Treatments		SEM	P-value
	NS	SS		
				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

Item	Treatments			P-value		
	NS	SS	SEM	Sup ¹	Day ²	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 ³	0.33	0.27	0.042	0.377	<0.001	0.206
βHB, mmol/L ³	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)

Figures

Figure 1

Average daily gain during pre- and post-partum period. Asterisks (*) indicate significant differences between treatments ($P<0.10$). NS: Nonsupplemented cows, SS: Supplemented cows.

Figure 2

Glucose plasma concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P<0.10$).

Figure 3

Triglycerides serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P<0.10$).

Figure 4

Total cholesterol serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P < 0.10$).

Figure 5

HDL (a), LDL (b) and VLDL (c) serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P < 0.10$).

Figure 6

Total protein (a), albumin (b) and globulin (c) serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P < 0.10$).

Figure 7

Creatinine serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P<0.10$).

Figure 8

Blood urea nitrogen (BUN) concentrations during pre- and post-calving. Numbers followed by asterisks (*) are significantly different between treatments ($P<0.10$).

Figure 9

Non-esterified fatty acids (NEFA) serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P<0.10$).

Figure 10

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

Figure 11

IGF-1 (a) e Insulin (b) serum concentrations during pre- and post-calving. Different letters indicate significant differences between days ($P<0.10$).

Figure 12

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