UNIVERSIDADE FEDERAL DE VIÇOSA

ROBERT TEIXEIRA DA PAIXÃO

EFFECT OF CREEP-FEEDING SUPPLEMENTATION ON GROWTH, AND METABOLIC AND REPRODUCTIVE CHARACTERISTICS OF NELLORE HEIFERS

VIÇOSA - MINAS GERAIS
2021

ROBERT TEIXEIRA DA PAIXÃO

EFFECT OF CREEP-FEEDING SUPPLEMENTATION ON GROWTH, AND METABOLIC AND REPRODUCTIVE CHARACTERISTICS OF NELLORE HEIFERS

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

Adviser: Cláudia Batista Sampaio

VIÇOSA – MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

Paixão, Robert Teixeira da, 1995-

P149e
2021

Effect of creep-feeding supplementation on growth, and metabolic and reproductive characteristics of Nellore heifers / Robert Teixeira da Paixão. – Viçosa, MG, 2021.

30 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.

Orientador: Cláudia Batista Sampaio.

Dissertação (mestrado) - Universidade Federal de Viçosa.

Referências bibliográficas: f. 20-24.

1. Bovinos de corte. 2. *Bos indicus*. 3. Novilhos - Crescimento. 4. Puberdade. I. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. II. Título.

CDD 22. ed. 636.213

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB6 2523

ROBERT TEIXEIRA DA PAIXÃO

EFFECT OF CREEP-FEEDING SUPLEMENTATION ON GROWTH, AND METABOLIC AND REPRODUCTIVE CHARACTERISTICS OF NELLORE HEIFERS

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

APPROVED: June 1, 2021.

Assent:

Robert Teixeira da Paixão
Author

Cláudia Batista Sampaio
Adviser

DEDICATION

This work is dedicated to all the people that had encouraged and supported me in one way or another along the way.

To my family, Cláudio, Carmem Lucia, Luciene and Cláudio, who always gave me all the support to keep going on this journey.

And to my friends, Mariana, Jardeson, Dhones, Paulo, Pamela, Cris, Thamys, Adailton, Saulo for all their companionship and friendship during the master´s period.

This is for you!

AKNOWLEDGEMENTS

To God for being my base and my guide in all the decisions that I had to make along this journey of life.

To the Universidade Federal de Viçosa, especially to the Animal Science Department, for making this course possible.

To my parents, Cláudio and Carmem Lucia, for being my biggest supporters. I love you.

To my brothers Cláudio and Luciene, for supporting me and who always believed in my potential as professional and as a person. I love you

To the National Council for Scientific and Technological Development (CNPq) for granting the Master's scholarship.

This work was carried out with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) - Financing Code 001.

To professor Cláudia Batista Sampaio, for her confidence, opportunities, teachings and excellent guidance.

To professor Marcos Marcondes, for his availability to do the statistical analysis.

To the employees of the UEPE-GC sector and Líbia for all the collaboration and execution of this project.

To fellow trainees from UEPE-GC sector who contributed with enormous help throughout the experiment.

To my friends, that the Animal Science Department at UFV made it possible for me to have, especially Jardim, Dhones, Paulo, Cris, Rizi, Pames, Saulo, Dadá, Flávia, Mariana, Thamys and all the others that were not mentioned s2. Thanks for the outings, conversations and friendships. You were fundamental in this process.

To everyone who, directly or indirectly, contributed to this work, thank you so much.

ABSTRACT

PAIXÃO, Robert Teixeira, M.Sc., Universidade Federal de Viçosa, June, 2021. **Effect of creep-feeding supplementation on growth, and metabolic and reproductive characteristics of Nellore heifers.** Adviser: Cláudia Batista Sampaio.

Objectives were to evaluate the effects of supplementation at pre-weaning phase on growth performance, metabolic, and reproductive characteristics of Nellore heifers. Forty-two Nellore female calves (average age = 100 ± 25 d; average initial body weight [BW] = 113.4 ± 16.6 kg) were randomly assigned to one of two treatments (n = 21) at the pre-weaning phase: control = heifers received mineral mix supplementation; and supplemented = heifers received 6 g/kg BW of a concentrate supplement. During the post-weaning phase, all heifers received 6 g/kg BW of a concentrate supplement. The experiment lasted 320 d. Supplemented heifers had greater average daily gain (ADG) than control heifers at pre-weaning phase and, consequently, were heavier at weaning and final of growing phase ($P < 0.05$). However, pre-weaning supplementation did not influence ($P > 0.05$) the body measurements or body weight at the end of growing period. Greater ($P < 0.05$) rib fat was observed in supplemented heifers. Concentrations of metabolites and the and conception rates were not affected ($P > 0.05$) by pre-weaning supplementation. Thus, supplementing heifers at the pre-weaning phase improved growth performance at weaning and body adiposity however, without additional performance or metabolic characteristics.

Keywords: Beef cattle. *Bos indicus*. Growth. Heifer. Puberty.

RESUMO

PAIXÃO, Robert Teixeira, M.Sc., Universidade Federal de Viçosa, junho de 2021. **Efeito da suplementação com creep-feeding sobre o crescimento e características metabólicas e reprodutivas de novilhas Nelore**. Orientadora: Cláudia Batista Sampaio.

Os objetivos foram avaliar os efeitos da suplementação na fase de pré-desmame sobre o desempenho de crescimento, características metabólicas e reprodutivas de novilhas Nelore. Quarenta e duas bezerras Nelore fêmeas (idade média = 100 ± 25 dias; peso corporal inicial médio [PC] = 113,4 ± 16,6 kg) foram aleatoriamente designados para um de dois tratamentos (n = 21) na fase de pré-desmame: controle = as novilhas receberam suplementação com mistura mineral; e suplementadas = as novilhas receberam 6 g / kg de PC de suplemento concentrado. Durante a fase pós-desmame, todas as novilhas receberam 6 g / kg de PC de suplemento concentrado. O experimento durou 320 dias. Novilhas suplementadas tiveram maior ganho médio diário (GMD) do que novilhas controle na fase pré-desmame e, consequentemente, foram mais pesadas ao desmame ($P < 0,05$). No entanto, a suplementação pré-desmame não influenciou ($P > 0,05$) as medidas corporais ou peso corporal ao final do período de crescimento. Maior ($P < 0,05$) gordura nas costelas foi observada nas novilhas suplementadas, enquanto as concentrações gerais de progesterona foram maiores ($P < 0,05$) nas novilhas controle. As concentrações de metabólitos e a taxa de concepção não foi afetada ($P > 0,05$) pela suplementação pré desmame. Assim, a suplementação de novilhas na fase pré-desmame melhorou o desempenho de crescimento à desmama e a adiposidade corporal, no entanto, sem desempenho adicional ou nas características metabólicas.

Palavras-chave: Bovinos de corte. Bos *indicus*. Crescimento. Novilha. Puberdade.

SUMMARY

Introduction

In a tropical system, grass forage represent the main feedstuff for beef cattle, as it is one of the most practical and economical production system (Latawiec et al., 2014). However, seasonality effects frequently pose a challenge to sustain the forage nutritional value throughout the year. PEDREIRA *et al.* (2009) observed a drop on forage mass and protein content of approximately 45 and 10 % in fall when compared to the summer values of *Brachiaria decumbens* pasture under Southeast Brazil tropical conditions. Thus, due the quantitative and qualitative variation over time of forage supply, their nutritional value may be reduced, which may result at late age at puberty and first pregnancy of beef heifers (Moreira et al., 2015).

Nutritional status and body development has a great impact on the age at puberty of beef heifers (Cardoso et al., 2014). There is a reasonably widespread agreement that heifers should achieve about 55 to 65% of their mature body weight at first service and that this should have increased to 85 to 90% by first calving. Moreover, nutritional interventions that improve nutrient utilization, body fat accretion, and increase circulating concentrations of hormones that facilitate the puberty process are known to hasten puberty attainment in heifers (Cooke et al., 2008). Thus, in intensive production systems aiming reduced age at first calving, ensuring high body weight gain rates in heifers during both pre- and post- weaning phases would be essential for improving their fertility rate and, consequently, their reproductive efficiency.

However, research regarding the positive effects of supplementation strategies on growth performance and the onset of puberty in grazing beef heifers has concentrated its efforts in specific situations of the production cycle, verifying, in most cases, their effects only after weaning. Less attention has been given to the impact of early life management on timing of puberty. Nevertheless, in a review, GASSER (2013) has stated that precocious puberty has been induced in heifers with increased growth rates at pre-weaning phase and in those submitted to early weaning and subsequent feeding a high concentrate diet.

In this context, supplementation strategies during the pre-weaning phase, such as the *creep-feeding* system, must be developed. The *creep-feeding* system usually provides greater weaning weight and, consequently, reduces the duration of the post-weaning phase (Paulino, 1999; Carvalho et al., 2019). Previous research on programing the onset of puberty in beef heifers indicated that beef heifers that were properly fed during the early stages of development presented a better physiological status, which resulted in precocious puberty (Cardoso et al., 2014). The authors of this study postulated that their results reflected the changes in the hypothalamic-pituitary axis of the female calves (during the 4th and 7th months of age), generating memory cells and greater synthesis of receptors for hormones related to reproduction, triggering the puberty process. Furthermore, Gasser et al. (2006) suggested that the follicular development of the ovary and estradiol production would be intense in heifers that were early weaned and fed a high concentrate diet to induce precocious puberty.

Although the exact physiological mechanisms of precocious puberty are still not fully understood, previous studies have suggested that leptin-sensitive cells synthesized in adipocytes and hypothalamus play a critical role in this process (Garcia et al., 2003; Maciel et al., 2004). In fact, changes in body composition usually result in endocrine responses. In most cases, greater deposition of adipocytes results in a greater production of leptin, which is involved in signaling the start of the reproductive phase (Zhang et al., 1995). Therefore, heifers with greater weight gain rates due proper nutrient supplementation since early life may have greater leptin synthesis and may be able to express precocious puberty. Thus, we hypothesized that supplementation of Nellore heifers at pre-weaning phase would improve their growth performance, metabolic, and reproductive characteristics in relation to heifers supplemented only at post-weaning phase. Therefore, the objectives were to evaluate the effects of supplementation at pre- weaning phase on growth performance metabolic, and reproductive characteristics of Nellore heifers.

Materials and Methods

Ethical considerations, animals handling, experimental design, and supplements

The experiment was conducted at Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais (20° 45′ S 42° 52′ W) from February to December 2018. The institutional animal care and use committee approved all procedures involving animals (Protocol number 58).

Forty-two Nellore female nursing calves (average age = 100 ± 25 d; average initial body weight [BW] = 113.4 ± 16.6 kg) were used in the current study. All heifers were accompanied by their respective dams until weaning at 7 months of age. The experimental period was divided into two phases: pre- and post- weaning. Pre-weaning phase lasted 140 days, from February to June, and the post-weaning phase lasted 180 days, from July to December. The concentrate supplement offered at pre-weaning phase was offered in creep-feeding feeder on pasture, where only the calves in this group had access in fed.

The experiment was run as a completely randomized design. The heifers were randomly assigned to one of two treatments (n = 21 heifers per treatment) at the pre-weaning phase: control = heifers received mineral mix supplementation; and supplemented = heifers received 6 g/kg BW daily of a concentrate supplement (Carvalho et al., 2019). During the post-weaning phase, all heifers received 6 g/kg BW daily of a concentrate supplement. The concentrate supplements contained 18.9 and 28.3% crude protein (on a dry matter [DM] basis), in the pre- and post-weaning phases, respectively. The supplement was offered once a day at 1000 h (Table 1) in a common feeder for each group in *a creep-feeding* system.

An experimental area of 32 ha with ten paddocks was assigned to the 42 heifers divided in eight paddocks measuring 3.2 ha each, two paddocks with six heifers and six paddocks with five heifers. The soil was covered uniformly with the *Urochloa decumbens* grass and equipped with water dispensers and feeders. Each experimental group grazed one paddock. Every 7 days, the animals were rotated between the paddocks. This was done to control any paddock effects on the treatments (pasture availability, water dispenser and feeder locations, relief, shading, and so on). Average rainfall and temperature values during the experimental period were 5.01 mm and 14.6 ºC, respectively.

Experimental procedures and sampling

The height at withers (from the highest point of the shoulder blade to the ground was recorded at beginning, weaning, and at the end of the growing period with a height stick. The heifers were weighed at the beginning and the end of both pre- and post-weaning phases to monitor average daily gain (ADG) in each growing phase. At weaning and the end of the experiment, rib eye area (REA) and rib fat thickness of the area between the 13th and 14th ribs, and rump fat thickness were measured by ultrasound scan (Aloka SSD 500; 3.5- MHz linear probe; Aloka Co. Ltd., Wallingford, CT, USA). Vegetable oil was used to ensure adequate acoustic contact. Images were analyzed in the BioSoft Toolbox® II for Beef (Biotronics Inc., Ames, Iowa, USA) (SILVA *et al.*, 2017).

Four blood samples were collected at the beginning of supplementation phase, at weaning, at the end of growing period, and at pre-breeding (before submitting heifers to the fixed-time artificial insemination [FTAI] protocol) to evaluate the concentrations of glucose, urea, total protein, albumin, globulin and leptin where the globulin was calculated by subtracting albumin from total protein. In addition, three blood samples were also taken on 45, 30, and 15 d prior to the fixed-time artificial insemination protocol (FTAI) to evaluate the progesterone concentrations. Blood samples were collected by jugular venous puncture at 0800 h. The samples were collected in 8.5 mL tubes with clot activator and gel for serum separation (BD Vacutainer® SST II Plus, São Paulo, Brazil). After collection, the samples were centrifuged at 3,600 ×g for 15 min at 4°C, and then serum were transferred to 2 mL microcentrifuge tubes and immediately frozen (-20°C) until further analysis.

At d 390 of age, all heifers were submitted to an artificial insemination procedure (FTAI). For this, all heifers received an intravaginal monodose implant (Primer®, Tecnopec, Brazil) containing 1.9 g progesterone (CIDR-B®, Pfizer Animal Health, Brazil) plus 2 mg of estradiol benzoate (Estrogin®, Farmavet, Brazil). Nine days after the beginning of the FTAI, the implant was removed and 1.5 mL of equine chorionic gonadotropin (Ecegon®, Biogeneses Bago, Brazil) plus 2.0 mL of prostaglandin F2 alpha (Estron®, Agener União, Brazil) was administered. Twenty-four hours after the implant removal (d 400), 1 mL of estradiol benzoate (RIC-BE®, Tecnopec, Brazil) was administrated. Pre-ovulatory follicular diameter was measured by ultrasonography (DP-2200Vet® with a 7.5-MHz linear-array transrectal transducer; Mindray) 48 h after the implant removal (d 401). Heifers that presented pre-ovulatory follicular diameter larger than 11 mm were artificial inseminated. Conception rate were measured 30 days after the artificial insemination. A second FTAI was performed in heifers that did not present a positive conception diagnosis in the first FTAI, following the same procedure.

Forage samples were randomly taken every 28 days to evaluate the forage mass per hectare. In each paddock, five forage samples were randomly selected using a metal square (0.5 × 0.5 m) and cut at approximately 1 cm above the soil. Additionally, every 28 days, a hand-plucking sample collection was performed to evaluate the chemical composition of the forage consumed by the animals. All samples were oven-dried (55 °C), grounded with 1- and 2-mm knife mills (Willye® TE-680), and proportionally subsampled to a composite sample per paddock and per period for further analysis.

Laboratory analyses and calculations

Supplement and forage samples were processed to pass a 1-mm sieve which were analyzed with regards dry matter (DM; dried overnight at 105ºC; method G003/1), ash (complete combustion in a muffle furnace at 550°C; method M-001/1), and N (Kjeldahl procedure; method N-001/1) and neutral detergent fiber (NDFap) corrected for ash and protein (method F-002/1) contents according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012). The neutral detergent fiber (NDF) contents were evaluated using a heat-stable α-amylase and omitting sodium sulfite according to Mertens et al. (2002).

Forage samples were procedure thought a 2-mm screen sieve and evaluated for indigestible NDF (iNDF) content using non-woven bags (100 g/m²), for 288-h in situ incubation (Valente et al., 2011). The potentially digestible DM (pdDM; Paulino et al., 2008) was calculated by the following equation:

$$pdDM = 0{:}98 \times (100 - NDF) + (NDF - iNDF)$$

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

Concentrations of glucose (ref. number K082), urea (ref. number K056), total protein (ref. number K031), and albumin (ref. number K040) were measured using kits from Bioclin Diagnostics (Bioclin® Quibasa Química Básica Ltda, Belo Horizonte, Brazil). These metabolites previously described were analyzed following the manufacturer's instructions in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China). Concentrations of leptin and progesterone concentrations were measure by radioimmunoassay kit (125/RIA, ICN Pharmaceuticals, Inc, EUA) and quantified by in PerkinElmer-wizard -1470 automatic - gamma counter (Laboratory of Endocrinology/Physiology of Domestic Animals, UNESP, Araçatuba campus, Sao Paulo, Brazil).

Statistical analysis

Data were analyzed according to the model:

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

where Y_{ijk} is the response measured in animal k within paddock j submitted to treatment I, μ is the general constant, C_i is the treatment (with or without creep feeding) effect (fixed), $P_{(i)j}$ is the effect of paddock j nested to treatment i (random), and $\varepsilon_{(ij)k}$ is the random error assumed to NID (0, σ^2_ε).

All analyzes were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). When repeated measurements were performed, the choice of the best (co)variance matrix was based on Akaike information criterion with correction. The denominator degrees of freedom were estimated by using the Kenward-Roger method. For all comparisons and tests, 0.05 was adopted as the critical level of probability for a type-I error.

Results

Growth performance and body measurements

There was a treatment × period interaction for body weight ($P = 0.026$) and ADG ($P < 0.001$) of heifers (Figure 1). During the pre-weaning phase, supplemented heifers had greater ADG than control heifers (supplemented = 0.82 kg/d and control = 0.72 kg/d) and, consequently, were heavier at weaning ($P < 0.05$). However, pre-weaning supplementation did not influence ($P > 0.05$) the body weight at the end of growing period, however the ADG at post-weaning phase was greater ($P = 0.019$) for control heifers.

No treatment × period interactions ($P \geq 0.053$) were observed for height at withers (Figure 2, a) or body weight:height at withers ratio (Figure 2, b). While there was no main effect of treatment on height at withers and body weight:height at withers ratio ($P \geq 0.060$), there was a main effect of period ($P < 0.001$) for height at withers and body weight:height at withers ratio. Both variables, height at withers and body weight:height at withers ratio of heifers, increased ($P < 0.05$) over period, regardless of treatment.

No treatment × period interaction was observed ($P \geq 0.145$) for REA, rib fat, and rump fat (Table 2). No main effect of treatment ($P \geq 0.200$) was observed for REA or rump fat. However, rib fat was greatest ($P < 0.017$) in supplemented heifers compared to control heifers. Moreover, greater ($P < 0.001$) REA, rib fat, and rump fat were observed at the end of the growing period compared to the values observed at weaning, regardless of treatments.

Metabolic and reproductive characteristics

No treatment × period interactions ($P \geq 0.093$) were observed for concentrations of glucose (Figure 3, a), urea (Figure 3, b), total protein (Figure 3, c), albumin (Figure 3, d), globulin (Figure 3, e) or leptin (Figure 3, f). Moreover, no main effect of treatment was observed ($P \geq 0.162$) for glucose, urea, total protein, albumin, and leptin concentrations. Nevertheless, there was a main effect of period ($P < 0.001$) for concentrations of glucose, urea, albumin, and leptin. Furthermore, heifers had increasing urea concentrations ($P < 0.05$) until the end of growing period. At the pre-breeding period, urea concentrations decreased, being intermediate and different from other periods ($P > 0.05$).

There was an effect of treatment ($P = 0.020$) and period ($P = 0.001$) on progesterone concentrations. Progesterone concentrations were greatest ($P = 0.002$) in control heifers when compared to supplemented heifers. Moreover, progesterone concentrations increased ($P =0.001$) over period, regardless of treatment.

Discussion

The age at puberty in beef heifers is controlled by genetic and environmental factors. Among these factors, body development and nutritional status has a great impact (Cardoso et al., 2014). There is a widespread agreement that heifers should achieve a minimal body development by first breeding, which correspond to approximately 55 to 65% of their mature body weight. Moreover, it is also preconized that heifer's body weight should have increased to 85 to 90% of mature body weight by first calving. Furthermore, nutritional strategies that are able to improve body fat content, and increase circulating concentrations of hormones that induce the puberty process, such as leptin, are known to hasten puberty attainment in heifers (Cooke et al., 2008). Usually, the objective is to improve conception rates to first breeding at 13 to 15 months of age by having a greater proportion of heifers reaching puberty by 30 to 45 d before the breeding season (Gasser, 2013). Thus, improving body development of heifers since early life would be mandatory for improving their fertility rate and, consequently, their reproductive efficiency.

Indeed, many different attempts to stimulate the early onset of puberty in beef heifers have been described in the literature (Patterson et al., 1991; Amstalden et al., 2014; de Almeida et al., 2019). However, most strategies were based on nutrient supplementation to improve heifers' nutritional status and growth performance after weaning (Gasser, 2013). Less attention has been directed to the effects of early maturation period on reproductive development of beef heifers, which take place at the pre-weaning period (between birth and approximately 8 months of age, usually). Nevertheless, there are instances in the literature that have indicated that accelerated growth in heifers at early stages of development (up to 9 months of age) would be more positive on the timing of puberty (Gasser, 2013; Amstalden et al., 2014).

Previous research on programing the onset of puberty in beef heifers indicated that feeding early weaned heifers a high-concentrate diet from about 4 to 7 months of age resulted in greater gain rates and better physiological status, which resulted in precocious puberty (Gasser et al., 2006b; Gasser et al., 2006a; Cardoso et al., 2014). Similarly, Rodriguez-Sanchez et al. (2015) demonstrated that age at puberty is negatively correlated with pre-weaning gain rate (up to approximately 6 months of age) but not after weaning. Therefore, these studies demonstrated the positive effect of improved growth rate during the early life on heifers' development.

These results from previous studies contributed to our hypothesis that supplementing grazing beef heifers since the pre-weaning phase could improve their growth performance, metabolic, and reproductive characteristics. This hypothesis was partially confirmed in the current study as supplemented heifers during the pre-weaning phase presented greater ADG and body weight at weaning. Moreover, the improved performance at pre-weaning was reflected in an overall increased rib fat instead of muscle deposition, as no difference on overall REA was observed among supplemented and control heifers. However, pre-weaning supplementation had no effect on ADG during the post-weaning phase, body measurements, and body weight at the end of the growing period. Similar results were found by Reis et al. (2015), that concluded that providing creep-feeder access to nursing heifers did not impact post-weaning growth and body composition variables, including Longissimus muscle development and subcutaneous fat deposition via hyperplasia (adipocyte density) or hypertrophy (adipocyte area).

Concentrations of glucose, urea, total protein, globulin and albumin are indicators generally associated with ruminant energy and protein metabolism, thereby useful to characterize nutritional status of heifers in this study. For example, increased concentrations of glucose, urea, total protein, and albumin have been reported in growing cattle receiving increasing levels of supplementation (Silva et al., 2017; de Almeida et al., 2019; Ortega et al., 2020), as a result of increased nutrient intake and absorption. However, our results did not follow the same pattern. No treatment × period interactions or main effect of treatment were observed for any of the metabolites evaluated. Our results suggest that difference on nutrient intake between treatments might not be substantial enough to promote changes in blood metabolites. In fact, a moderate level of supplementation was utilized in the current study (6 g/kg BW). Furthermore, forage quality was higher than usual for tropical pastures (Table 1) due favorable weather conditions during this experiment (average rainfall and temperature values were 5.01 mm and 14.6 ºC, respectively).

Nevertheless, reduced glucose and increased leptin concentrations were observed in heifers over the course of experimental period. The reduced concentrations of glucose over time observed in this study may be a result of the gradual decrease in dam's milk production and total interruption of milk intake due weaning and consequently, lower participation or absence of milk in heifers total diet (Henriques et al., 2011; Ortega et al., 2020). Glucose concentration decreased as calves grew older, indicating a shift in the source of nutrients (Khan et al., 2011). Furthermore, the time-related overall increased leptin concentrations may be due to the increase in body fat deposition, as leptin is a key metabolic signal of nutritional/metabolic status synthesized mainly by fat cells (Zieba et al., 2005). Unexpectedly, however, the concentration of leptin did not differ among treatments at any of periods, as an overall effect of supplementation on adiposity was observed. These results are in contrast with the results described by Cardoso et al. (2014), where an increase in leptin concentrations as heifers became fatter was reported.

It is well known that increasing nutritional level impacts age at puberty. However, the exact mechanisms and pathways involved in the nutritional effect on the onset of puberty have not been completely elucidated. Previous studies have suggested that a group of factors and hormones such as IGF-1, endogenous opioids, neuropeptide Y, leptin, insulin, and other, are regulated by nutritional status and serves as messengers modulating the GnRH secretion and release (Garcia et al., 2003; Zieba et al., 2005; Amstalden et al., 2014). Such pathway regulation results in a stimulation of gonadotropins secretion and release leading to ovulation (Roa et al., 2010; Amstalden et al., 2014). Thus, as the heifers reach sexual maturity, the ovaries become functional and increase progesterone production (Rodriguez-Sanchez et al., 2015). In this context, we expected that supplemented heifers would present greater follicular diameter and progesterone concentrations compared with control heifers. However, such effect of pre-weaning supplementation was not observed in the current study. Control and supplemented heifers did not differ on follicular diameter, and conception rates at all periods evaluated. Moreover, control heifers presented greater overall progesterone concentrations, indicating that pre-weaning supplementation had no positive effect on triggering the early onset of puberty in those animals. Similarly to our results, Nepomuceno et al. (2017) and Silva et al. (2017) have reported no effect of pre-weaning supplementation on early puberty onset. Nonetheless, supplemented heifers presented a numerically higher conception rate, and hence a gestation possible, in relation to control heifers (Figure 4). From a practical standpoint, supplementation at pre-weaning phase represented five more pregnant heifers at the end of the breeding season, that could suggest program of puberty, and pregnancy by supplementation in growing period. Therefore, more studies in this area need to be carried out, using a larger number of animals.

In summary, average daily gain at pre-weaning phase, body weight at weaning, and rib fat but not body measurements, concentrations of metabolites and hormones, and conception rates at FTAI were positively affected by pre-weaning supplementation in Nellore heifers. In conclusion, supplementing heifers at the pre-weaning phase improved growth performance at weaning and body adiposity; however, with no additional benefit on overall growth performance, metabolic, or reproductive characteristics.

References

Amstalden, M., R. C. Cardoso, B. R. C. Alves, and G. L. Williams. 2014. Reproduction Symposium: Hypothalamic neuropeptides and the nutritional programming of puberty in heifers. J. Anim. Sci. 92:3211–3222. doi:10.2527/jas.2014-7808.

Cardoso, R. C., B. R. C. Alves, L. D. Prezotto, J. F. Thorson, L. O. Tedeschi, D. H. Keisler, C. S. Park, M. Amstalden, and G. L. Williams. 2014. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. J. Anim. Sci. 92:2942–2949. doi:10.2527/jas.2014-7713.

Carvalho, V. V., M. F. Paulino, E. Detmann, S. C. V. Filho, S. A. Lopes, L. N. Rennó, C. B. Sampaio, and A. G. Silva. 2019. A meta-analysis of the effects of creep-feeding supplementation on performance and nutritional characteristics by beef calves grazing on tropical pastures. Livest. Sci. 227:175–182. doi:10.1016/j.livsci.2019.07.009. Available from: https://doi.org/10.1016/j.livsci.2019.07.009

De Almeida, D. M., M. I. Marcondes, L. N. Rennó, L. H. Pereira Silva, L. S. Martins, D. E. C. Marquez, F. A. Castaño Villadiego, F. Velez Saldarriaga, J. D. C. Franco, D. P. S. Moreno, F. H. de Moura, and M. F. Paulino. 2019. Nutritional planning for Nellore heifers post-weaning to conception at 15 months of age: performance and nutritional, metabolic, and reproductive responses. Trop. Anim. Health Prod. 51:79–87. doi:10.1007/s11250-018-1662-z.

Garcia, M. R., M. Amstalden, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2003. Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age. J. Anim. Sci. 81:261–268. doi:10.2527/2003.811261x.

Gasser, C. L. 2013. Considerations on puberty in replacement beef heifers. J. Anim. Sci.

91:1336–1340. doi:10.2527/jas2012-6008.

Gasser, C. L., E. J. Behlke, D. E. Grum, and M. L. Day. 2006a. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. J. Anim. Sci. 84:3118–3122. doi:10.2527/jas.2005-676.

Gasser, C. L., D. E. Grum, M. L. Mussard, F. L. Fluharty, J. E. Kinder, and M. L. Day. 2006b. Induction of precocious puberty in heifers I: Enhanced secretion of luteinizing hormone. J. Anim. Sci. 84:2035–2041. doi:10.2527/jas.2005-636.

Henriques, L. T., S. de C. Valadares Filho, M. A. Fonseca, P. V. R. Paulino, E. Detmann, and R. F. D. Valadares. 2011. Avaliação de modelos não-lineares e da relação do consumo voluntário de vacas primíparas e de bezerros com a curva de lactação de vacas Nelore. Rev. Bras. Zootec. 40:1287–1295. doi:10.1590/S1516-35982011000600018. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982011000600018&lng=pt&tlng=pt

Khan, M. A., D. M. Weary, and M. A. G. Von Keyserlingk. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. J. Dairy Sci. 94:3547–3553. doi:10.3168/jds.2010-3871. Available from: http://dx.doi.org/10.3168/jds.2010-3871

Latawiec, A. E., B. B. N. Strassburg, J. F. Valentim, F. Ramos, and H. N. Alves-Pinto. 2014. Intensification of cattle ranching production systems: Socioeconomic and environmental synergies and risks in Brazil. Animal. 8:1255–1263. doi:10.1017/S1751731114001566.

Maciel, M. N., D. A. Zieba, M. Amstalden, D. H. Keisler, J. P. Neves, and G. L. Williams. 2004. Leptin Prevents Fasting-Mediated Reductions in Pulsatile Secretion of Luteinizing Hormone and Enhances Its Gonadotropin-Releasing Hormone-Mediated Release in Heifers. Biol. Reprod. 70:229–235. doi:10.1095/biolreprod.103.021345.

Moreira, H. L., M. E. Buzanskas, D. P. Munari, É. B. Canova, R. B. Lôbo, and C. C. P. de Paz.

2015. Reproductive Traits Selection in Nelore Beef Cattle. Ciência e Agrotecnologia. 39:355–362. doi:10.1590/s1413-70542015000400006.

Nepomuceno, D. D., A. V Pires, M. V. C. Ferraz, M. V Biehl, J. R. S. Gonçalves, E. M. Moreira, and M. L. Day. 2017. E ff ect of pre-partum dam supplementation , creep-feeding and post- weaning feedlot on age at puberty in Nellore heifers. Livest. Sci. 195:58–62. doi:10.1016/j.livsci.2016.11.008. Available from: http://dx.doi.org/10.1016/j.livsci.2016.11.008

Ortega, R. M., M. F. Paulino, E. Detmann, L. N. Rennó, D. S. Moreno, D. C. Márquez, D. M. de Almeida, L. P. de Melo, and F. H. Moura. 2020. Nutritional strategies for heifers under grazing system: productive and nutritional performance, metabolic profile and ovarian activity. Trop. Anim. Health Prod. 52:1013–1022. doi:10.1007/s11250-019-02095-7. Available from: http://link.springer.com/10.1007/s11250-019-02095-7

Patterson, D. J., L. R. Corah, J. R. Brethour, M. F. Spire, J. Higgins, G. H. Kiracofe, J. S. Stevenson, and D. D. Simms. 1991. Evaluation Of Reproductive Traits In Bos Taurus. J. Anim. Sci. 69:2349–2361.

Paulino, M. F. 1999. Estratégias de suplementação para bovinos de corte. In: Symposium of beef cattle production. p. 93–139.

Paulino, M. F., E. Detmann, and S. C. Valadares Filho. 2008. Bovinocultura Funcional nos trópicos. In: Symposium of beef cattle production. Universidade Federal de Viçosa, Viçosa. p. 275–305.

Pedreira, M. dos S., O. Primavesi, M. A. Lima, R. Frighetto, S. G. de Oliveira, and T. T. Berchielli. 2009. Ruminal methane emission by dairy cattle in Southeast Brazil. Sci. Agric. 66:742–750. doi:10.1590/s0103-90162009000600004.

Reis, M. M., R. F. Cooke, B. I. Cappellozza, R. S. Marques, T. A. Guarnieri Filho, M. C. Rodrigues, J. S. Bradley, C. J. Mueller, D. H. Keisler, S. E. Johnson, and D. W. Bohnert.

2015. Creep-feeding to stimulate metabolic imprinting in nursing beef heifers: Impacts on heifer growth, reproductive and physiological variables. Animal. 9:1500–1508. doi:10.1017/S1751731115000828. Available from: http://dx.doi.org/10.1017/S1751731115000828

Roa, J., D. García-Galiano, J. M. Castellano, F. Gaytan, L. Pinilla, and M. Tena-Sempere. 2010. Metabolic control of puberty onset: New players, new mechanisms. Mol. Cell. Endocrinol. 324:87–94. doi:10.1016/j.mce.2009.12.018. Available from: http://dx.doi.org/10.1016/j.mce.2009.12.018

Rodriguez-Sanchez, J. A., A. Sanz, and C. Tamanini. 2015. Metabolic , endocrine , and reproductive responses of beef heifers submitted to different growth strategies during the lactation and rearing periods Metabolic , endocrine , and reproductive responses of beef heifers submitted to different growth strategie. J. Anim. Sci. 93:3871–3885. doi:10.2527/jas2015-8994.

Silva, Aline Gomes, M. F. Paulino, L. da Silva Amorim, L. N. Rennó, E. Detmann, F. H. de Moura, M. R. Manso, P. H. Silva e Paiva, R. E. M. Ortega, and L. P. de Melo. 2017. Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre- and post-weaning. Trop. Anim. Health Prod. 49:707–715. doi:10.1007/s11250-017-1248-1.

Silva, A. G., M. F. Paulino, L. Silva Amorim, L. N. Rennó, E. Detmann, F. H. Moura, M. R. Manso, P. H. Silva e Paiva, R. E. M. Ortega, and L. P. Melo. 2017. Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre- and post-weaning. Trop. Anim. Health Prod. 49:707–715. doi:10.1007/s11250-017-1248-1. Available from: http://link.springer.com/10.1007/s11250-017-1248-1

Valente, T. N. P., E. Detmann, A. C. de Queiroz, S. de Campos Valadares Filho, D. I. Gomes, and J. F. Figueiras. 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Rev. Bras. Zootec. 40:2565–2573. doi:10.1590/S1516-35982011001100039.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1995. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425–432.

Zieba, D. A., M. Amstalden, and G. L. Williams. 2005. Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest. Anim. Endocrinol. 29:166–185. doi:10.1016/j.domaniend.2005.02.019.

Table 1 Composition of forage and supplements offered in the pre- and post-weaning phases

	Pre-weaning			Post-weaning	
	Forage	Treatment		Forage	Treatment
		Control	Supplemented		Supplemented
Ingredients (% dry matter)					
Ground corn	-	-	70.3	-	20.47
Soybean meal	-	-	24.8	-	46.47
Wheat bran	-	-	3.08	-	29.71
Mineral mix[a]	-	100	-	-	-
Mineral mix[b]	-	-	1.81	-	-
Mineral mix[c]	-	-	-	-	3.34
Chemical composition (% dry matter)					
Dry matter	60.6	100	88.3	39.9	87.9
Organic matter	89.0	-	95.0	91.5	91.8
Crude protein	8.34	-	18.9	10.0	28.3
apNDF[d]	73.4	-	13.8	63.9	17.4
Undigestible neutral detergent fiber	31.6	-	-	26.3	-
Potentially digestible dry matter (kg/ha)	2256	-	-	5969	-

[a]Composition: 15% of Ca, 11,4% of P, 18,6% of Na, 28,6% of Cl, 0,34% Zn, 0,36% of S, 0,02% of Co, 0.18% of Cu, 0,16% of Mn, 2.7 × 10-3% of Se, and 0,03% of I.

[b]Composition: 0,48% of Ca, 0,06% of P, 0,08% of Na, 0,13% of Cl, 2.2 × 10-3% of Zn, 2.3 × 10-3% of S, 1.0 × 10-4% of Co, 1.1 × 10-3% of Cu, 1.0 × 10-3% of Mn, 2.0 × 10-5% of Se, 2.0 × 10-4% of I; 7019,9979 UI of vitamin A, 1934,9994 UI of vitamin D3, 1.0 × 10-3% of vitamin B1, 6.6 × 10-3% of vitamin B2, 4.0 × 10-5% of K3, 2.0 × 10-8% of vitamin B6, 3.0 × 10-4% of vitamin B12, 3.5 × 10-4% of Niacin, 4.0 × 10-7% of Biotin, 7.0 × 10-6% of Folic acid, 5.0 × 10-4% of BHT, and 2.0 × 10-7% of Calcium pantothenate. [c]Composition: 0,45% of Ca, 0,34% of P, 0,56% of Na, 0,86% of Cl, 3.0 × 10-3% of Zn, 4.0 × 10-3% of S, 2.0 × 10-3% of Co, 2.0 × 10-3% of Cu, 2.0 × 10-4% of Mn, and 3.0 × 10-3% of I.

[d]Neutral detergent fiber corrected to ash and protein.

Table 2. Effect of pre-weaning supplementation on body measurements of heifers at weaning and at the end of growing period

Item	Treatment[a]		SEM[b]	P value[c]		
	Control	Supplemented		Treat	Period	Treat × Period
Rib eye area (cm^2)						
Weaning	32.4	35.2	0.991	0.200	<0.001	0.145
End of growing period	43.2	43.6				
Rib fat (mm)						
Weaning	0.87	1.16	0.066	0.017	<0.001	0.738
End of growing period	1.34	1.62				
Rump fat (mm)						
Weaning	1.70	1.74	0.092	0.988	<0.001	0.423
End of growing period	2.46	2.41				

[a]Treatment: Control = heifers were not supplemented in the pre-weaning phase; Supplemented = heifers were supplemented at the pre-weaning phase.
[b]Standard error of mean.
[c]Treat = effect of supplementation in the pre-weaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

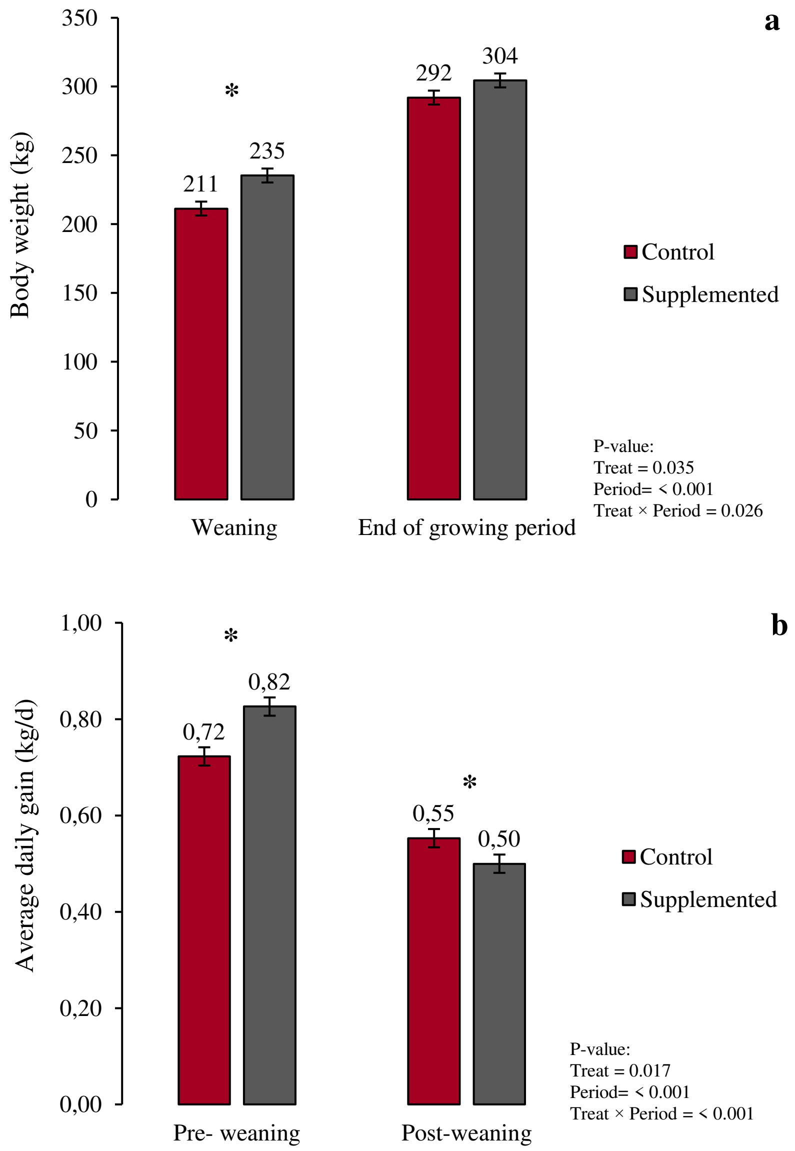

Figure 1. Effect of pre-weaning supplementation on body weight (a) and average daily gain (b) at weaning and at the end of growing period of Nellore heifers. Treatments: Control = heifers without concentrate at pre-weaning phase; Supplemented = heifers with concentrate at the pre-weaning phase. Treat = effect of supplementation at the pre-weaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

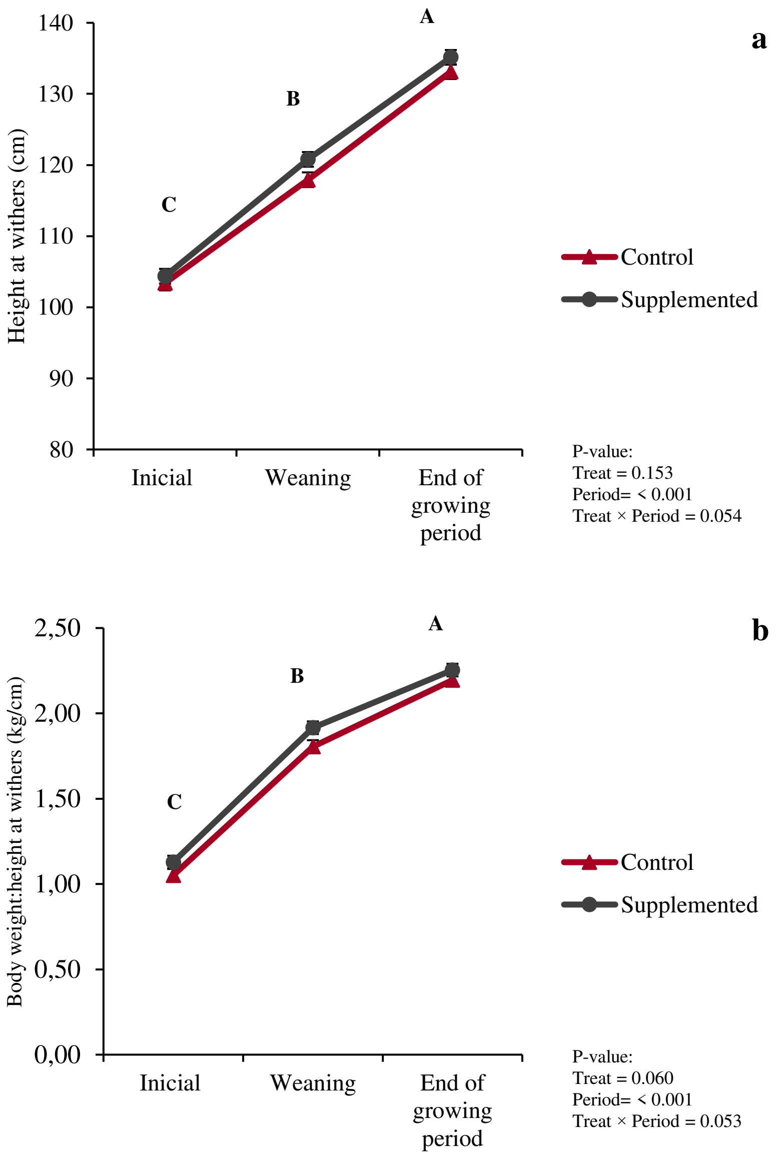

Figure 2. Effect of pre-weaning supplementation on height at withers (a) and body weight:height at withers ratio (b) at beginning (initial), weaning and at the end of growing period of Nellore heifers. Treatments: Control = heifers without concentrate at pre-weaning phase; Supplemented = heifers with concentrate at the pre-weaning phase. Treat = effect of supplementation at the pre-weaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

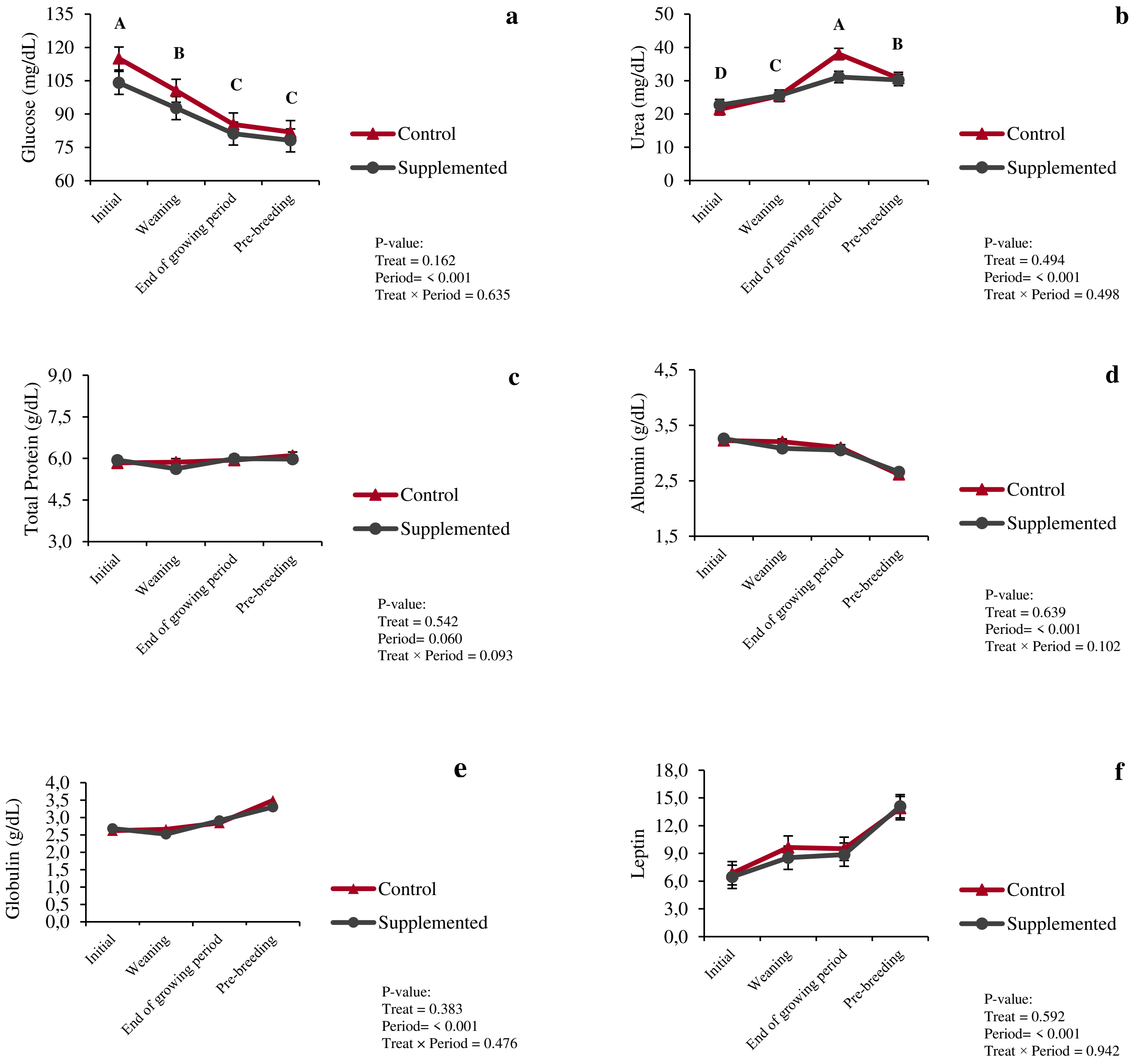

Figure 3. Effect of pre-weaning supplementation on concentrations of glucose (a), urea (b), total protein (c), albumin (d), globulin (e) and leptin (f) at beginning (initial), weaning, the end of growing period, and pre-breeding periods of Nellore heifers. Treatments: Control = heifers without concentrate at pre-weaning phase; Supplemented = heifers with concentrate at the pre-weaning phase. Treat = effect of supplementation at the pre-weaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

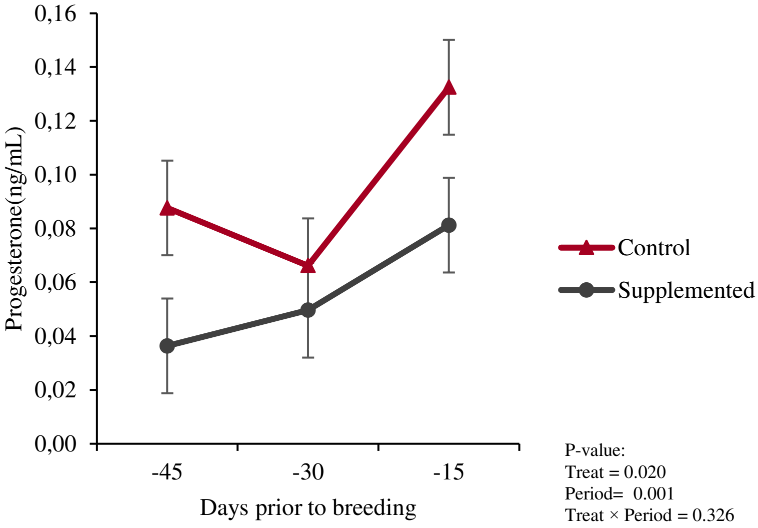

Figure 4. Effect of pre-weaning phase supplementation on progesterone blood concentrations of heifers on 45, 30, and 15 d prior to breeding. Treat = effect of supplementation at the pre-weaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

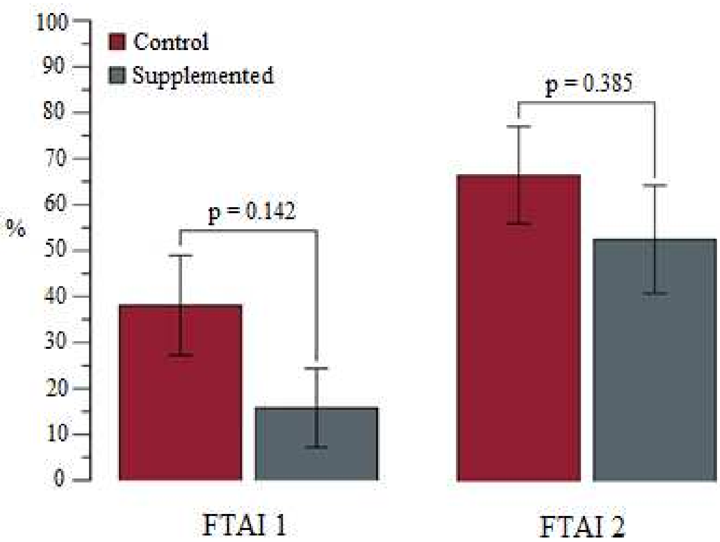

Figure 5. Effect of pre-weaning phase supplementation on percentage of heifers presenting conception rates at fixed time artificial insemination 1 (FTAI 1) and 2 (FTAI 2) of heifers.