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Effect of Creep Feeding Supplementation on Growth Performance and Metabolic Characteristics of Nellore Heifers

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Abstract: The objective of this paper is to evaluate the effects of creep feeding supplementation during the preweaning phase on the growth performance and metabolic characteristics of Nellore heifers. Forty-two female Nellore calves (age = 100 ± 25 d; initial body weight (BW) = 113.4 ± 16.6 kg) were randomly assigned to the following treatments: control, where calves received mineral mix supplementation (n = 21); supplemented in creep feeding, where calves received 6 g/kg BW of a concentrate supplement (n = 21) during a period of 140 d. In the postweaning phase, all heifers received 6 g/kg BW of a concentrate supplement during a period of 210 d. Supplemented heifers had a greater average daily gain (ADG) than control heifers during the preweaning phase and, consequently, were heavier at weaning and at the end of the growing phase ($p < 0.05$). However, preweaning supplementation did not influence ($p > 0.05$) the body measurements or BW at the end of the growing period. Greater ($p < 0.05$) rib fat was observed in supplemented heifers. Concentrations of metabolites were not affected by preweaning supplementation ($p > 0.05$). Thus, supplementing heifers in the preweaning phase improved growth performance of weaning and body adiposity.

Keywords: beef cattle; growth; leptin; metabolism; puberty

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1. Introduction

The quantitative and qualitative factors of forage supply in tropical conditions [1] may result in advanced age at puberty and first pregnancy of beef heifers [2]. The availability and quality of forage and voluntary intake affects body development, which has a major impact on the age of puberty of these animals [3]. There is a reasonably widespread agreement that heifers should achieve about 55 to 65% of their mature body weight (BW) to be capable of conception and that their BW must increase to 85 to 90% that of mature BW at calving to achieve adequate reproductive efficiency [4].

Gasser [5] found that precocious puberty could be induced in heifers with increased growth rates during the preweaning phase and in those submitted to early weaning and subsequent feeding of a high-concentrate diet. Supplementation causes changes in the hypothalamic–pituitary axis of female calves between four and seven months of age, generating memory cells and greater synthesis of receptors for reproduction-related hormones related to reproduction, thus triggering the puberty process [3]. Furthermore, Gasser et al. [6] reported that follicular development in the ovary and estradiol production were intense in heifers that were weaned early 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, synthesized in adipocytes, and

leptin-sensitive cells in the hypothalamus play a critical role in this process [7–9]. In fact, changes in body composition usually result in endocrine responses. Heifers with greater weight gain rates due to proper nutrient supplementation since early life may show greater leptin synthesis and may attain precocious puberty. In most cases, a higher number of adipocytes results in a greater production of leptin, which is involved in signaling the start of the reproductive phase [10]. In this sense, the use of creep feeding leads to a higher weaning weight and, consequently, reduces the duration of the postweaning phase [11,12].

However, research regarding the positive effects of supplementation strategies on growth performance and the onset of puberty in grazing beef heifers has mainly focused on the postweaning phase. Less attention has been given to the impact of early life management on the onset of puberty.

Thus, we hypothesized that creep feeding supplementation of Nellore heifers during the preweaning phase would improve their growth performance and metabolic status in relation to heifers supplemented only during the postweaning phase. Therefore, the objective was to evaluate the effects of supplementation during the preweaning phase on growth performance and the metabolic and reproductive characteristics of Nellore heifers.

2. Materials and Methods

The experiment was conducted at the Research, Teaching, and Extension Unit for Beef Cattle at the Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. The animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of the UFV (Protocol number: 58/2018).

2.1. Animals, Experimental Treatments

Forty-two female Nellore calves (age = 100 ± 25 d; initial BW = 113.4 ± 16.6 kg) were investigated, accompanied by their respective dams until weaning at 7 months of age (240 d). The experimental period was divided into two phases: pre- and postweaning. The preweaning phase lasted 140 days (February to June), and the postweaning phase lasted 210 days (July to January)—in the last 60 days of this period (breeding phase), all heifers were submitted to a fixed-time artificial insemination (FTAI) protocol.

The experiment was carried out as a completely randomized design. The heifers were randomly assigned to eight groups with five or six animals each. Then, the following two treatments were randomly assigned to the different groups, with four groups and 21 heifers per treatment: control—mineral mix ad libitum during the preweaning phase; supplemented—6 g/kg BW daily of a concentrate supplement during the preweaning phase in creep-feeding feeder on pasture, to which only the calves had access. After weaning, the dams were moved out the experimental paddocks and the animal groups were kept as defined at the beginning of the experiment, and all heifers received 6 g/kg BW daily of a concentrate supplement (Table 1) offered once a day at 10:00.

Table 1. Composition of forage and supplements offered in the pre- and postweaning phases.

	Preweaning			**Postweaning**	
	Forage [5]	**Treatment**		**Forage** [5]	**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 [1]	-	100	-	-	-
Mineral mix [2]	-	-	1.81	-	-
Mineral mix [3]	-	-	-	-	3.34

Table 1. *Cont.*

	Preweaning			Postweaning	
	Forage [5]	Treatment		Forage [5]	Treatment
		Control	Supplemented		Supplemented
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 [4]	73.4	-	13.8	63.9	17.4

[1] 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. [2] 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; 7019.10 UI of vitamin A, 1934.10 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. [3] 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. [4] Neutral detergent fiber corrected to ash and protein. [5] The chemical composition of the forage was quantified in the hand-plucked samples.

During the rearing phase, eight paddocks measuring 3.2 hectares (ha) each were used; two paddocks contained six heifers and six paddocks contained five heifers. The soil was uniformly covered with the *Urochloa decumbens* grass and the paddocks were equipped with water dispensers and feeders. Each experimental group grazed one paddock using the continuous stocking method.

2.2. Experimental Procedures and Sampling

The heifers were weighed at the beginning and the end of the pre- and postweaning phases to quantify the average daily gain (ADG) in each growth phase. The height at the withers (from the highest point of the shoulder blade to the ground) was recorded at the beginning, weaning, and end of the growing period with a measuring stick. During weaning and at the end of the experiment, the *Longissimus dorsi* muscle area (LMA) 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). Images were analyzed with the BioSoft Toolbox® II for Beef (Biotronics Inc., Ames, IA, USA).

Blood samples were collected at the beginning of the supplementation phase, at weaning, at the end of the growing period, and prior to breeding (before submitting heifers to the FTAI) to evaluate the concentrations of glucose, urea, total protein, albumin, globulin, and leptin. 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 FTAI to measure progesterone concentrations. Blood samples were collected by jugular venous puncture at 08:00. The blood 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 3600× *g* for 15 min at 4 °C, after which the serum was transferred to 2 mL microcentrifuge tubes and immediately frozen (−20 °C) until further analysis.

During the breeding phase, i.e., during the last 60 days of the growing phase and when the heifers were 14 months old, all animals 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 start 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 F2alpha (Estron®, Agener União, Brazil) was administered. Twenty-four hours after implant removal, 1 mL of estradiol benzoate (RIC-BE®, Tecnopec, Brazil) was administered. Preovulatory follicular diameter was measured by ultrasonography (DP-2200Vet® with a 7.5-MHz linear array transrectal transducer; Mindray) 48 h after the implant removal. Heifers that presented

preovulatory follicular diameters larger than 11 mm were artificially inseminated. Conception rates were measured 30 days FTAI. Heifers that were not pregnant received a second artificial insemination (FTAI2), 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 m × 0.5 m) and cut at approximately 1 cm above the soil. Additionally, every 28 days, a hand-plucked sample was collected to evaluate the chemical composition of the forage consumed by the animals. All samples were oven-dried (55 °C) and milled to pass through a 1 mm screen sieve. Samples were pooled based on paddock and period for further analysis. Supplement samples were taken monthly and milled in a similar way.

2.3. Laboratory Analyses and Calculations

Supplement and forage samples were milled to pass through a 1 mm screen sieve and were analyzed for the following parameters using the procedures proposed by the National Institute for Animal Science and Technology (INCT-CA) [13]: DM (dried overnight at 105 °C; INCT-CA method G003/1), ash (complete combustion in a muffle furnace at 550 °C; INCT-CA method M-001/1), nitrogen (Kjeldahl procedure; INCT-CA method N-001/1), and neutral detergent fiber corrected for ash and protein (apNDF; INCT-CA method F-002/1) using a heat-stable α-amylase and omitting sodium sulfite [14].

Glucose and urea concentrations were quantified by an enzymatic–colorimetric method (K082 and K056, respectively; Bioclin Quibasa®, Belo Horizonte, Minas Gerais, Brazil), and total protein and albumin were analyzed by a colorimetric method (K031 and K040, respectively, Bioclin Quibasa®, Belo Horizonte, Minas Gerais, Brazil). An automated biochemical analyzer (Mindray BS 200E, Shenzhen, China) was used for all previously mentioned analyses. Leptin and progesterone concentrations were measured with a radioimmunoassay kit (125/RIA, ICN Pharmaceuticals, Inc., St. Charles, MO, USA) and quantified by a PerkinElmer Wizard 1470 Gamma Counter (Laboratory of Endocrinology/Physiology of Domestic Animals, UNESP, Araçatuba Campus, Sao Paulo, Brazil).

2.4. Statistical Analyses

Data were analyzed according to the following model:

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

where Y_{ijk} is the response measured in animal k within paddock j submitted to treatment I, μ is the general constant, S_i is the fixed effect of supplementation during the preweaning period (with or without), $P_{(i)j}$ is random the effect of paddock j nested to treatment i, and $\varepsilon_{(ij)k}$ is the random error assumed to be NIID (0, $\sigma^2{}_\varepsilon$).

All analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). After each analysis of variance, the random error was estimated for all observations and its normal distribution was checked using the Shapiro–Wilk test. For all response variables, we found that random errors could be studied according to the normal distribution ($p > 0.05$). 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 using the Kenward–Roger method. For all analyses, 0.05 was adopted as the critical level of probability for type-I error.

3. Results

3.1. Growth Performance and Body Measurements

There was a treatment × period association with the BW ($p < 0.03$) and ADG ($p < 0.01$) of heifers (Figure 1a). During the preweaning period, supplemented heifers had greater ADG than controls ($p < 0.05$). Consequently, supplemented heifers were heavier at weaning ($p < 0.05$). However, preweaning supplementation did not influence ($p > 0.05$) BW at the

end of growing period, because ADG during the postweaning period was greater ($p < 0.02$) for control heifers (Figure 1b).

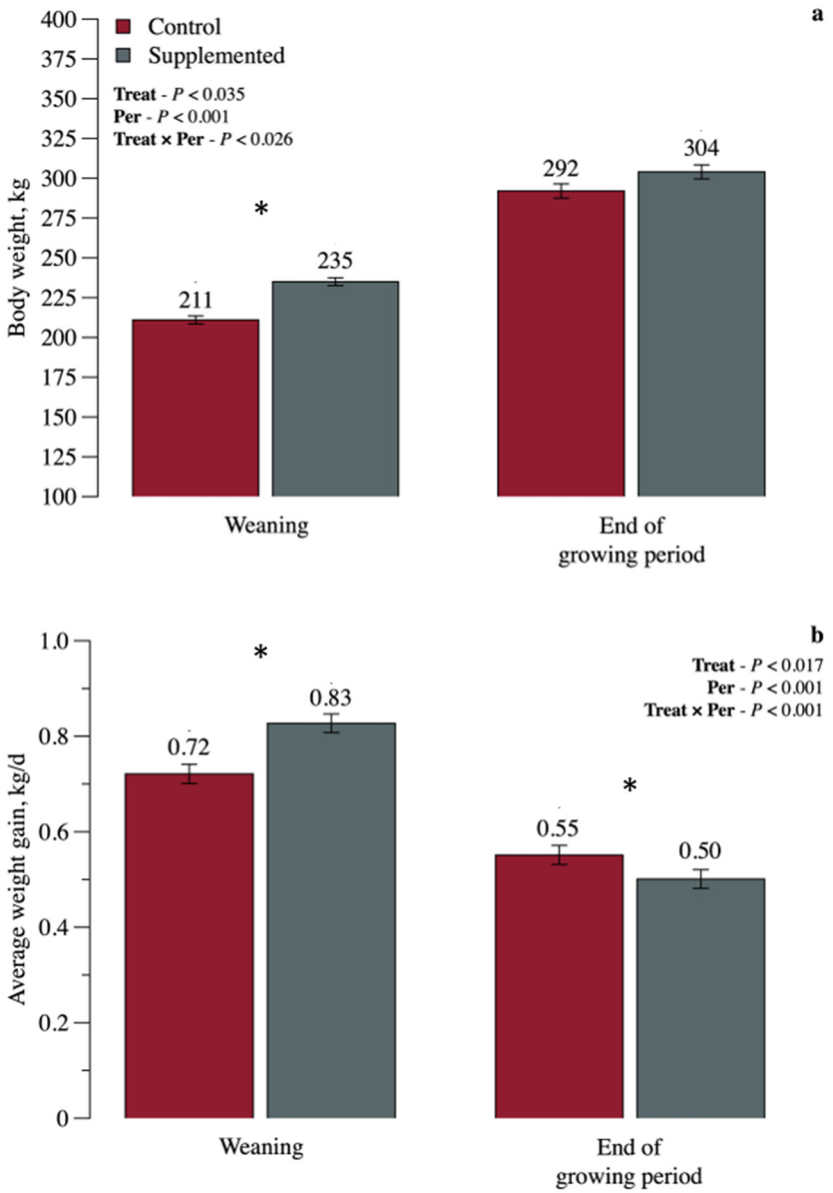

Figure 1. Effect of creep feeding supplementation on body weight (**a**) and average daily gain (**b**) during weaning and at the end of the growing period of Nellore heifers. Treatments: Control = heifers without concentrate at preweaning phase; Supplemented = heifers with supplementation in creep feeding. Treat = effect of supplementation during the preweaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period. (*) Means of treatments within a period differ at $p < 0.05$.

Height at the withers (Figure 2a) and the ratio of body weight/height at the withers (Figure 2b) were not affected by treatment × period or supplementation ($p > 0.05$). However, both variables increased ($p < 0.01$) as the heifers got older, regardless of treatment.

No treatment × period interaction was observed ($p > 0.14$) for LMA, rib fat, and rump fat (Table 2). Moreover, there no supplementation effect was observed for LMA ($p > 0.20$) or rump fat ($p > 0.98$). However, on average, rib fat was thicker ($p < 0.02$) in supplemented heifers. LMA, rib fat, and rump fat values were higher ($p < 0.01$) at the end of the growing period compared with the values observed at weaning, regardless of supplementation.

3.2. Metabolic and Reproductive Characteristics

No treatment × period interactions ($p > 0.09$) were observed for blood concentrations of glucose, urea, total protein, albumin, globulin, or leptin (Figure 3). Moreover, a treatment effect was observed ($p \geq 0.05$) for glucose, urea, total protein, albumin, and leptin concentrations. Nevertheless, all variables shown in Figure 3 were affected by period ($p < 0.01$). Blood glucose decreased ($p < 0.05$) from the beginning of the experiment to the end of the growing period and remained stable until the pre-breeding evaluation. Blood urea peaked ($p < 0.05$) at the end of growing period. On average, blood protein remained almost stable throughout the experiment, presenting a slightly lower concentration during weaning ($p < 0.05$). Blood albumin presented the highest values ($p < 0.05$) at the beginning of the experiment and decreased during weaning and the end of the growing period, with a further decrease at the pre-breeding evaluation, while the blood globulin moved in the opposite way when compared with albumin. Leptin increased up to weaning ($p < 0.05$), remained stable until the end of the growing period ($p > 0.05$), and presented a further increase at the pre-breeding evaluation ($p < 0.05$).

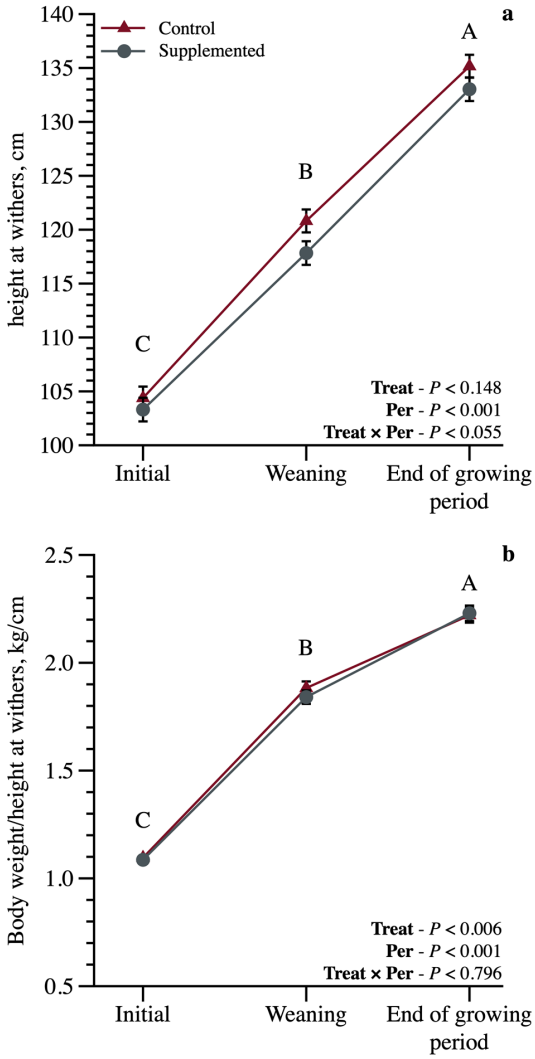

Figure 2. Effect of creep feeding supplementation on height at withers (**a**) and body weight/height at withers ratio (**b**) at beginning (initial), weaning, and end of the growing period of Nellore heifers. Treatments: Control = heifers without concentrate at preweaning phase; Supplemented = heifers with supplementation in creep feeding. Treat = effect of supplementation during the preweaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period. A, B, C: means with different superscripts differ between periods at $p < 0.05$.

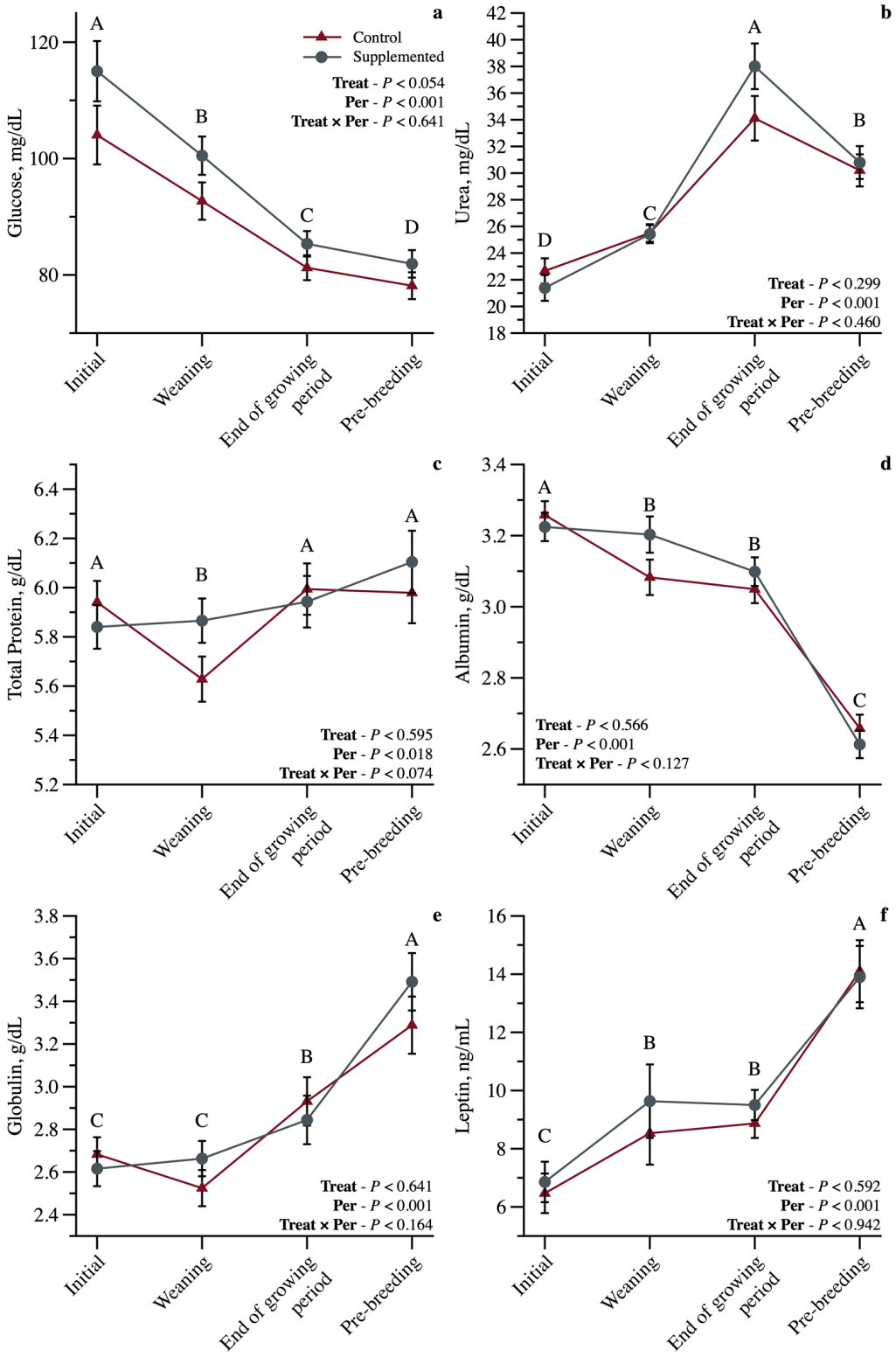

Figure 3. Effect of creep feeding supplementation on concentrations of glucose (**a**), urea (**b**), total protein (**c**), albumin (**d**), globulin (**e**), and leptin (**f**) at beginning (initial), weaning, and end of the growing period, and during the pre-breeding periods, of Nellore heifers. Treatments: Control = heifers without concentrate at preweaning phase; Supplemented = heifers with supplementation in creep feeding. Treat = effect of supplementation during the preweaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period. A, B, C: means with different superscripts differ between periods at $p < 0.05$.

Table 2. Effect of creep feeding supplementation on body measurements of heifers during weaning and at the end of the growing period.

Item	Treatment [1]		SEM [2]	*p*-Value [3]		
	Control	Supplemented		Treat	Period	Treat × Period
Longissimus dorsi muscle 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				

[1] Treatment: Control = heifers were not supplemented in the preweaning phase; Supplemented = heifers with supplementation in creep feeding. [2] Standard error of mean. [3] Treat = effect of supplementation in the preweaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period.

There was an effect of treatment ($p < 0.02$) and period ($p < 0.01$) on blood progesterone concentration (Figure 4). On average, blood progesterone concentration was higher ($p < 0.01$) in control heifers. In addition, progesterone concentrations increased ($p < 0.05$) from 30 days to 15 days before breeding time.

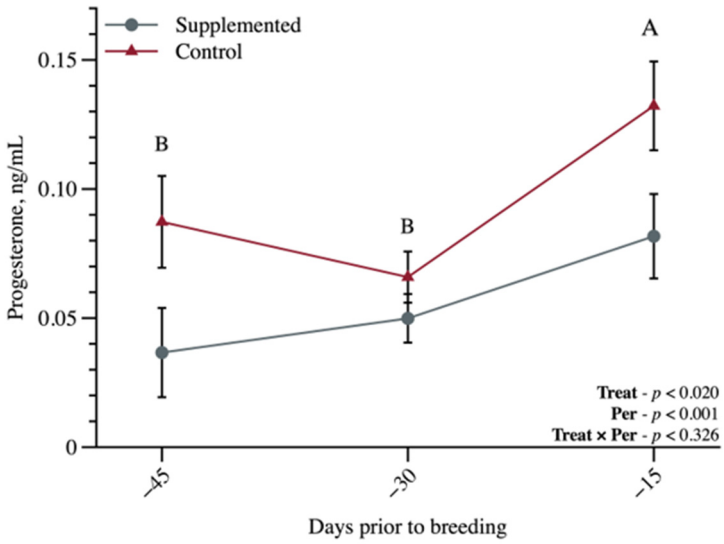

Figure 4. Effect of creep feeding supplementation on progesterone blood concentrations of heifers on 45, 30, and 15 d prior to breeding. Treat = effect of supplementation during the preweaning phase; Period = effect of period; Treat × Period = effect of the interaction between supplementation and period. A, B: means with different superscripts differ between periods at $p < 0.05$.

After the FTAI protocol during breeding, the conception rates were calculated as a practical result. Heifers that received supplementation showed conception rates of 40% in FTAI 1 and 70% in FTAI 2, while the control heifers had conception rates of 15% in FTAI 1 and 55% in FTAI 2.

4. Discussion

The association of treatment × period with BW and ADG showed that the supplementation improved the body development in the preweaning phase, which can be used to hasten the onset of puberty. The age at puberty in beef heifers is controlled by genetic and environmental factors. Among these factors, body development and nutritional status have a major impact [3,15]. Previous research on programing the onset of puberty in beef heifers indicated

that feeding early-weaned heifers a high-concentrate diet from about four to seven months of age resulted in higher daily gain rates and better physiological status, which resulted in precocious puberty [3,6,16,17].

Even though there was no effect of preweaning supplementation on BW at the end of the growing period, we observed that height at the withers and the ratio of body weight/height at the withers of heifers increased over the study period. In addition, the fat deposition observed at the rib fat and the increase in LMA, rib fat, and rump fat show that the supplementation may have switched cell metabolism to a more efficient use of nutrients [5] and growth hormone circulation [18]. Animals that were not supplemented reached a similar weight at the end of the growing phase, probably due to an improvement in feed efficiency as a compensatory gain effect [3]. This improvement in feed efficiency is followed by hormonal and metabolic changes that can direct nutrients toward body gain and composition [19].

In general, puberty traits—particularly the age and weight at first corpus luteum—respond to selection in tropical beef cattle [20]. Genetic characteristics in Zebu cattle that are associated with heifers' puberty and production traits include fat depth, rib fat, body condition, live weight, and *Longissimus dorsi* muscle area [21]. However, the relationship of growth and fatness with puberty traits is not expected to be linear, which is in agreement with our results that showed that, as measured by progesterone concentration, puberty traits were not achieved naturally at the end of the growing phase.

Blood concentrations of glucose, urea, total protein, globulin, and albumin are indicators that are generally associated with ruminant energy and protein metabolism, and are useful to characterize the nutritional status of heifers. For example, increased concentrations of glucose, urea, total protein, and albumin have been reported in growing cattle receiving increasing levels of supplementation [11] because of the increased nutrient intake and absorption. However, our results did not follow the same pattern. No treatment × period interactions or effect of treatments were observed for any of the evaluated metabolites. Our results suggest that the difference in nutrient intake between treatments might not have been substantial enough to promote changes in blood metabolites. The level of supplementation utilized in the current study was moderate (6 g/kg BW). Furthermore, forage quality was better than usual for tropical pastures (Table 1).

Reduced glucose and increased leptin concentrations were observed in heifers over the course of the experimental period. The reduced concentrations of glucose over time observed in this study may be the result of a gradual decrease in the dam's milk production; the total interruption of milk intake at weaning; and, consequently, lower participation or absence of milk in the heifers' total diet [22]. Glucose concentration decreased as calves grew older, indicating a shift in the source of nutrients [23]. Furthermore, the time-related overall increase in leptin concentrations may be due to an increase in body fat deposition, as leptin is a key metabolic signal of nutritional and metabolic status, which is mainly synthesized by fat cells [24].

Control heifers presented higher overall blood progesterone concentrations than supplemented heifers, indicating that preweaning supplementation had no effect on the onset of puberty. However, progesterone concentrations in both treatments indicated that puberty was not reached without a reproductive protocol. Previous studies have suggested that a group of factors and hormones such as IGF-I, endogenous opioids, neuropeptide Y, leptin, insulin, and others are regulated by nutritional status and serve as messengers modulating GnRH secretion and release [7,23,24]. Such pathway regulation stimulates gonadotropin secretion and release, leading to ovulation [24,25]. Thus, as the heifers reach sexual maturity, the ovaries become functional and increase progesterone production [26]. Progesterone production with the presence of a corpus luteum and after estrus can additionally lead to lower pregnancy losses after timed-artificial insemination in *Bos indicus* cattle, losses that are generally greater in primiparous cows [27]. In this context, we expected that supplemented heifers would have higher blood progesterone, which did not occur.

The effect of supplementation by creep feeding on metabolic imprinting, which is the biological responses to a nutritional intervention early in life that permanently alters

physiological outcomes later in life, is still under discussion [28], especially concerning puberty [29,30]. Similarly to our results, Nepomuceno et al. [31] and Silva et al. [12] did not find an effect of preweaning supplementation on early puberty onset. Nonetheless, supplemented heifers presented a numerically higher conception rate (70% vs. 55%), resulting in more pregnant heifers compared with the control group. From a practical standpoint, supplementation during the preweaning phase represented five more pregnant heifers at the end of the breeding season, which could suggest a program of puberty [3], and pregnancy by supplementation in the growing period. Therefore, more studies in this area need to be carried out using a larger number of animals.

5. Conclusions

Supplementation of Nellore heifers during the preweaning phase improves growth performance during weaning and body adiposity; however, it does not affect the natural onset of puberty.

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Institutional Review Board Statement: The animal management used in this study was approved by the Ethics Committee on Animal Use of the UFV (CEUAP—UFV; protocol: 58/2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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