Tropical Animal Health and Production (2023) 55:189
https://doi.org/10.1007/s11250-023-03588-2

REGULAR ARTICLES

Effect of growth rates on hormonal and pubertal status in Nellore heifers early weaned

M.V.C. Ferraz[1] · M.H. Santos[2] · G.B. Oliveira[2] · D.M. Polizel[7] · J.P.R. Barroso[2] · G.P. Nogueira[3] · V.N. Gouvea[4] · P.H.V. Carvalho[5] · J.S. Biava[6] · E.M. Ferreira[6] · A.V. Pires[2,6]

Received: 25 January 2023 / Accepted: 12 April 2023

Abstract

This study aimed to determine the effect of growth rates on the hormonal status and puberty onset. Forty-eight Nellore heifers were weaned at 3.0 ± 0.1 (means ± standard error of the mean) months old were blocked according to body weight at weaning (84 ± 2 kg) and randomly assigned to treatments. The treatments were arranged in 2 × 2 factorial according to the feeding program. The first program was high (H; 0.79 kg/day) or control (C; 0.45 kg/day) average daily gain (ADG) from 3rd to 7th month of age (growing phase I). The second program was also high (H; 0.70 kg/day) or control (C; 0.50 kg/day) ADG from the 7th month until puberty (growing phase II), resulting in four treatments: HH ($n = 13$), HC ($n = 10$), CH ($n = 13$), and CC ($n = 12$). To achieve desired gains, heifers in high ADG program were fed *ad libitum* dry matter intake (DMI), and the control group was offered around 50% of *ad libitum* DMI of high group. All heifers received a diet with similar composition. Puberty was assessed weekly by ultrasound examination, and the largest follicle diameter was evaluated every month. Blood samples were collected to quantify leptin, insulin growth factor-1 (IGF1) and luteinizing hormone (LH). At 7 months of age, heifers in high ADG were 35 kg heavier than the control. Heifers in the HH had greater DMI compared with CH in phase II. The puberty rate at 19 months old was greater in the HH treatment (84%) than in the CC (23%), but there was no difference between HC (60%) and CH (50%) treatments. Heifers from HH treatment had greater serum leptin concentration than others at 13 months old, and serum leptin was greater in HH compared with CH and CC at 18 months old. High heifers in phase I had greater serum IGF1 concentration than the control. In addition, HH heifers had a greater diameter of the largest follicle than CC. There was no interaction between phases and age in any variable relative to the LH profile. However, the heifers' age was the main factor that increased the frequency of LH pulse. In conclusion, increasing ADG was associated with greater ADG, serum leptin and IGF-1 concentration, and puberty onset; however, LH concentration was affected mainly by age of the animal. The increasing growth rate at younger age made heifers more efficient.

Keywords Beef cattle · IGF1 · Leptin · LH · Nutrition

✉ A.V. Pires
pires.1@usp.br

[1] Department of Animal Science, Federal University of Amazonas, Estrada Parintins Macurany, Jacareacanga, Parintins, AM 69152240, Brazil

[2] Department of Nutrition and Animal Production, College of Veterinary and Animal Science, University of São Paulo, Duque de Caxias North Avenue, n 225, Pirassununga, SP 13635-000, Brazil

[3] São Paulo State University (UNESP), Clovis Pestana 793, Araçatuba, SP 16050-680, Brazil

[4] Texas A&M AgriLife Research and Extension Center, Amarillo TX 79106, USA

[5] Department of Animal Science, University of California, Davis 95616, USA

[6] Department of Animal Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Pádua Dias Avenue, n 11, PO Box 09, Piracicaba, SP 13418-900, Brazil

[7] Department of Biology and Animal Science, Sao Paulo State University, Ilha Solteira, SP 15385000, Brazil

Published online: 04 May 2023

Introduction

Several studies have shown the effect of metabolic imprinting in heifers induced by increases in metabolic status around 3rd to 7th month of age, which accelerate the age at puberty (Gasser et al. 2006a; Cardoso et al., 2014; Moriel et al. 2014). Previous studies have reported that feeding energy-dense diets associated with early weaning advanced ovarian maturity in heifers, and increased the maximum diameter of dominant follicles, the length of follicular wave (Gasser et al. 2006b), and estradiol peak during follicular wave (Gasser et al. 2006c). Therefore, associating energy dense diets with early weaning may be an efficient nutritional strategy to eliminate the static phase during the prepubertal phase, anticipating the puberty of beef heifers (Gasser et al. 2006d).

Most of the studies assessing nutrition's effect on heifers' puberty were conducted using Taurine breeds. While there have been numerous studies examining puberty in *Bos indicus* cattle, there are few aware of any that specifically investigate the relationship between nutrition and precocious puberty (occurring before 18 months of age) in Zebu heifers. For instance, Johnston et al. (2009) explored the genetics of heifer puberty in two tropical beef genotypes in northern Australia, and found that Brahman heifers typically reach puberty after 2 years of age. The effect of nutritional strategies on Nellore heifers, which may be considered a non-precocious breed, is unknown.

Although it is common that Nellore heifers to reach puberty only after 24 months old (Nepomuceno et al. 2017; Ferraz Junior et al. 2018), previous studies have reported that Nellore heifers were considered precocious when heifers reached puberty earlier than 18 months old (Nepomuceno et al. 2017; Ferraz Junior et al. 2018), whereas a precocious Angus heifer was considered precocious when puberty occurred earlier than 10 months old (Gasser et al. 2006b; Gasser et al. 2006c; Gasser et al. 2006d). Therefore, there is the need to develop strategies to advance puberty in Nellore heifers. Moreover, it unknown how and when greater growth rates are more important in Nellore heifers prior to reach puberty. The hypothesis tested in this study was that augmenting the growth of Nellore heifers during their juvenile age would hasten the onset of puberty until they are below 18 months old. Therefore, the objective of the current study was to evaluate the effect of different growth rates by programmed feeding on the hormonal and puberty status of Nellore heifers.

Material and methods

The experiment was carried out at the Laboratory of Animal Nutrition and Reproduction, Department of Animal Science, "Luiz de Queiroz" College of Agriculture-ESALQ/USP, Piracicaba, SP, Brazil. The Animal Care and Use Committee from the University of São Paulo approved all animal procedures (# 7595290414).

Animals and experimental design

Forty-eight Nellore heifers were weaned at 3.0 ± 0.1 (means ± standard error of the mean) months old, daughters from the same sire with small expected progeny difference (within 10% percentile) for age at first calving according to Gensys (2013) catalog were used. Heifers were blocked according to initial body weight (BW) at weaning (84 ± 2 kg) and randomly assigned to the treatments. The treatments were arranged in 2 × 2 factorial with two feeding programs. The first was high (H) or control (C) average daily gain (ADG) from 3rd to 7th month of age (growing phase I). The second was also high (H) or control (C) ADG from 7th month of age to puberty (growing phase II), resulting in 4 different feeding treatments (HH, $n = 13$; HC, $n = 10$, CH, $n = 13$; CC, $n = 12$). To achieve desire ADG, heifers were fed at *ad libitum* dry matter intake (DMI) in high ADG and around 50% of *ad libitum* DMI in the control group.

All heifers received the same diet containing 19% and 14% of crude protein as dry matter basis in the first and second phases, respectively. Ingredients and chemical analyses of the experimental diets are shown in Table 1. Heifers were housed in collective pens (3 heifers/pen in *ad libitum* DMI and 2 heifers/pen in restricted DMI) and fed once a day using a forage wagon equipped with an electronic scale (Totalmix® 1500, Casale, São Carlos, SP, Brazil). Body weight was measured weekly from all heifers (Beckhauser idBeck 3.0®, Beckhauser, Paranavaí, PR, Brazil). To minimize fluctuations in ADG among heifers receiving the same treatment, heifers were re-grouped within the same treatment following each BW measurement and individual ADG analysis if the difference in ADG within the same pen exceeded 0.10 kg/day. We adjusted the amount of feed offered to each pen weekly, based on the ADG from the previous week. We collected diet samples for chemical composition analysis, and weighed feed refusals to calculate DMI. The experimental diets were formulated according to the NRC (1996) to promote an ADG of 1.0 kg/day in the *ad libitum* fed group. Diet total digestible nutrients were calculated based on the NRC (1996). Feed efficiency was determined as the ratio between ADG and DMI.

Chemical analyses and calculations

Samples of the diets and orts were ground in a Wiley mill (Marconi, Piracicaba, SP, Brazil) with a 1.0-mm sieve. The dry matter (DM) was determined after oven-drying the samples at 105 °C for 24 h according to the method of the

Table 1 Ingredients e chemical composition of experimental diets (DM basis)

Item	Phase I[a]	Phase II[b]
Ingredients[c] (% of DM)		
Cynodon haylage	21.5	–
Sugarcane bagasse	–	19.6
Whole cottonseed	–	10.1
Ground corn	57.5	59.1
Soybean meal	21.0	10.7
Urea	–	0.5
Analyzed chemical composition (% of DM)		
Dry matter (%, as fed basis)	78.6	75.5
Crude protein	19.5	14.1
Ether extract	4.3	5.9
Neutral detergent fiber	25.2	23.6
Ash	5.6	4.2
Total digestible nutrients[d]	79.1	78.7

[a]Phase I: from 3rd to 7th month of age

[b]Phase II: from 7th month to puberty or 19th month of age in non-pubertal heifers

[c]Mineral supplement was provided in mineral boxes *ad libitum*, and 30 ppm of sodium monensin (Rumensin 100®, Elanco Animal Health, São Paulo, SP, Brazil) was added to the diet

[d]Based on the tabular composition of ingredients (NRC 1996)

Association of Official Analytical Chemist (AOAC 1990; #934.01). Ash was determined by incinerating the samples in a muffle furnace at 550°C for 4 h (AOAC 1990; #942.05). Total nitrogen (N) concentration was determined using the Leco Tru Mac®/N (Leco® Corporation, St. Joseph, MI, USA; AOAC, #968.06) (AOAC 1990). Crude protein was obtained by multiplying the total N content by 6.25. Neutral detergent fiber was determined according to Van Soest et al. (1991), using thermostable alpha-amylase and sodium sulfite. Acid detergent fiber was determined (AOAC 1990; #954.01) using the Ankon 2000® Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA). The ether extract content was determined using a supercritical fluid extraction system (Leco TFE-2000®, Leco Corporation, St. Joseph, MI, USA).

Ultrasonography exam, sampling, and hormonal assay

Heifers weighing over 230 kg underwent weekly transrectal ultrasonography (US) examinations using the DP-2200 VET machine from Mindray, Shenzhen, China. These examinations were performed regularly until the onset of puberty and were used to evaluate the largest diameter of the dominant follicle and corpus luteum (CL). Additionally, blood samples were taken to determine circulating concentrations of progesterone (P4) if a CL was detected. Puberty was considered to have been reached when a CL was detected through US and confirmed by progesterone concentrations greater than 1.5 ng/mL (Cooke and Arthington, 2009). To assess the growth patterns of the follicles, we measured the largest follicle diameter using ultrasound exams every 2 days for 15 days each month. We then conducted a retrospective analysis to analyze the collected data. Blood samples were also collected to determine the leptin and insulin growth factor-1 (IGF1) concentrations at 3, 5, 7, 12, and 18 months old. Heifers were removed from the experiment as they reach puberty or at the 19th month of age when the experiment was finished. At 19 months of age, non-pubertal heifers received an intravaginal device of progesterone (1.9 g) previously used for 21 days. After 9 days, the device was removed, and 7 days later, the presence of CL on ovaries was checked via US.

All blood samples for P4, leptin and IGF1 determinations were collected from the coccygeal vein or artery in Vacutainer tubes (Greiner Bio-One Brasil, Americana, SP, Brazil) with inert serum separator gel. Serial blood from 5 heifers per treatment was collected at 15-min intervals for 12 h to assess LH pulsatility at 7, 9, 11, 13, 15, 16, 17, 18, and 19 months old. The blood samples were collected using indwelling jugular catheters connected to a hose circuit containing an anticoagulant. Prior to each collection, the anticoagulant in the circuit was discarded, after which 5 mL of blood was drawn and the anticoagulant was reintroduced into the system. Blood was allowed to clot for 24 to 48 h at 4°C. All blood samples collected were centrifuged for 15 min at 1800 × *g* (Refrigerated Centrifuge Excelsa®4, Mod.280R–Fanem®, São Paulo, SP, Brazil), and the harvested serum was frozen at −20 °C until further analysis.

The P4 concentrations were determined by a chemiluminescent assay using commercial IMMULITE® 1000 kits (Siemens Healthcare Diagnostics Products, Llanberis, UK). The P4 analysis sensitivity was 0.002 ng/mL, and the coefficient of variation (CV) for the high and low adjustments were 1.7 and 2.1%, respectively. Leptin concentrations were evaluated by a commercial radioimmunoassay kit (Multi-Species Leptin®, Millipore–XL–85k, Bedford, MA, USA), as reported previously (Ren et al. 2002). The intra- and inter-assay CV were 10.3 and 7.4%, respectively, and the sensitivity of the assay was 0.955 ng/mL.

As previously described, LH concentrations were determined in duplicate using radioimmunoassay (Bolt et al. 1990; Bolt and Rollins 1983). A highly purified LH (AFP8614B; National Hormone and Pituitary Program) was used for the iodinated tracer and reference standard preparation. The sensitivity of the assay was 0.05 ng/mL. The intra- and inter-assay coefficients of variation were 5.3% and 13.9%, respectively. The LH pulse, LH pulse amplitude, and mean LH concentrations were determined as described by

Goodman and Karsch (1980). Heifers were removed from blood sampling procedures when ovulation was confirmed.

Statistical analysis

Heifers were used as experimental units to evaluate the effects of the treatment on the pubertal status and hormonal concentrations. The pens were used as the experimental units to evaluate the effect of treatments on growth performance data. The continuous variables were analyzed for normality (Shapiro-Wilk) and homogeneity of variance (Welch test) before analysis with the mixed procedure of SAS (version 9.3; SAS Institute, Cary, NC, USA), using the model Yijk = μ + Ai + Bj + (AB)ij + D1 + εijk, where Yijk is the response variable; μ is the overall mean; Ai is the fixed effect of factor I; Bj is the fixed effect factor II; (AB)ij is the interaction effect between factor I and factor II; Dl is the random effect of the block; and εijk is the residual error term.

The mixed procedure also evaluated the leptin, IGF1, and LH concentrations using repeated measures, using the model Yijk = μ + Di + Bj + Eij + Pk + (BP)jk + (DP)ik + Eijk, where μ is the overall mean; Di is the fixed effect of treatments; Bj is the random effect of block; Eij is the residual error A; Pk is the fixed effect of time; (BP)jk is the random effect of block × time interaction; (DP)ik is the fixed effect of treatment x time interaction; and Eijk is the residual error B. The covariance matrices "compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, unstructured, banded, variance components, toeplitz, and heterogeneous toeplitz" were tested and selected based on the lowest value of Akaike's information criterion. Furthermore, a separate model was developed to assess the impact of puberty status (pubertal and non-pubertal heifers) on LH profile and the diameter of the largest follicle. The treatments compared heifers that underwent puberty before 18 months of age (classified as precocious heifers according to Ferraz et al. 2018 and Nepomuceno et al. 2017) to those that did not reach puberty (non-precocious heifers). It is important to note that all LH profiles were collected prior to the heifers reaching puberty and this study represents a retrospective analysis.

The percentage of pubertal heifers at the 19th month of age and the responses to the puberty induction protocol were analyzed by the Glimmix procedure using the binomial option, using a similar model for mixed model. The means were obtained by the lsmeans command, and mean comparisons were performed by the pdiff option. For all mixed and Glimmix procedures, the Satterthwaite approximation was used to determine the denominator degrees of freedom for the treatment effect. The age at puberty was analyzed by survival curve performed from the Lifetest procedure, and the Logrank test was used to determine the difference among curves.

Results

Female calves were weaned at 88 ± 2 days old, with a body weight of 84 ± 2 kg. Heifers with high ADG were around 40 kg heavier than the control at 7 months old (Table 2). As expected, the DMI was 60% less in heifers from control treatment in phase I. Likewise, phase II also induced a smaller (P <0.01) ADG in control heifers submitted to DMI restriction (Table 3). In phase II, a significant interaction was observed for DMI ($P < 0.05$) among the treatments, which HH treatment had a greater DMI compared to HC, CH, and CC. The CH group had greater ($P < 0.05$) DMI compared to HC and CC. The puberty rate at 19 months of age was affected ($P = 0.01$) by the treatments (Table 4; Fig. 1), in which HH treatment (84%) induced a greater ($P = 0.01$) proportion of pubertal heifers than the CC (23%). The puberty rate was similar in HC (60%) and CH (50%) treatments. In addition, the behavior of the puberty curves from HC and CH treatments were also similar throughout the whole experiment.

A significant interaction ($P < 0.01$) between treatments and age for serum leptin concentration was observed. At 13 months old, heifers from the HH treatment had higher serum leptin levels than those from other treatments, and this difference persisted at the 18th month compared to CH and CC (Fig. 2). In contrast, no interaction between treatments and age was observed for serum IGF1 concentration ($P = 0.68$). However, heifers with high ADG during phase I had higher serum IGF1 levels than those with control ADG at 5 and 7 months old ($P = 0.02$).

There was an interaction ($P < 0.01$) among phases and age in the diameter of the largest follicle, in which heifers from HH treatment had greater follicles compared to heifers from CC in most of the months evaluated during the experiment (Fig. 3). In addition, heifers that reached puberty had greater follicles than non-pubertal heifers at 11, 13, and 14 months.

Table 2 Performance of Nellore heifers in phase I (from 3rd to 7th month of age)

Variables	Treatments		*P*-value
	High	Control	
Initial age (day)	88 ± 2	88 ± 2	0.80
Initial BW (kg)	83 ± 2	86 ± 2	0.28
BW at 7th month of age (kg)	180 ± 4	141 ± 4	<.01
ADG (kg)	0.787 ± 0.03	0.448 ± 0.03	<.01
DMI (kg/day)	4.0 ± 0.10	1.6 ± 0.03	<.01
FE	0.19 ± 0.01	0.28 ± 0.01	<.01

Abbreviations: *ADG* average daily gain; *DMI* dry matter intake; *FE*, feed efficiency ratio between ADG and DMI. High, heifers submitted to high ADG with DMI *ad libitum*, ADG = 0.79 kg/day. Control, heifers submitted to restricted DMI, ADG = 0.45 kg/day. The means are reported with the standard error of the mean

Table 3 Performance of Nellore heifers in phase II (from the 7th month of age to puberty or 19th month of age in non-pubertal heifers)

Variables	Treatments				*P*-value		
	HH	HC	CH	CC	F1	F2	F1*F2
BW at 7th month of age (kg)	183 ± 5	180 ± 6	137 ± 6	145 ± 5	<.01	.	.
ADG (kg)	0.674 ± 0.02	0.524 ± 0.02	0.717 ± 0.02	0.520 ± 0.02	0.39	<.01	0.28
DMI (kg/day)	5.5^{a} ± 0.10	2.9^{c} ±0.05	4.7^{b} ±0.09	3.1^{c} ±0.06	<.01	<.01	0.05
FE	0.12^{c} ± 0.01	0.18^{a} ± 0.01	0.15^{b} ± 0.01	0.17^{ab} ± 0.01	0.02	<.01	<.01

Abbreviations: HH, heifers submitted to high average daily gain (ADG); dry matter intake (DMI) *ad libitum*) in phases I (from 3rd to 7th month of age) and II; *HC*, high ADG in phase I and control (50% DMI restricted) ADG in phase II; *CH*, control ADG in phase I and high ADG in phase II; *CC*, control ADG in the phases I and II; *BW*, body weight; *FE*, feed efficiency ratio between ADG and DMI. *F1*; the effect of phase I; *F2*, effect of phase II; *F1*F2*, interaction effect between F1 and F2. The means are reported with the standard error of the mean

Table 5 shows no significant interaction ($P > 0.05$) among phases and age in any variable related to the LH profile. However, heifers with control ADG had a higher ($P < 0.05$) frequency of LH pulses compared to those with high ADG in both phases at 15 and 16 months of age. Pubertal heifers ($n = 10$) exhibited a similar LH profile compared to non-pubertal heifers ($n = 10$), regardless of treatment (Fig. 4). Age was the main factor affecting LH profile, as shown in Fig. 5, with amplitude and maximum LH concentration increasing from the 7th to 9th month of age, while the frequency of LH pulses decreased. From the 9th to 17th month of age, the frequency of LH pulses gradually increased but dropped rapidly at the 18th and 19th month of age. The same pattern was observed for the diameter of the largest follicle. Figure 6 demonstrates an example of the decrease in the frequency of LH pulses, with the LH profile of a non-pubertal heifer with 179 kg and 390 kg of BW at 7 and 19 months of age, respectively.

At 19 months of age, non-pubertal heifers from HH, CH and HC treatments were heavier than pubertal heifers in their respective treatment. Only the heifers of the CC treatment (330 ± 9 kg) were lighter than pubertal heifers (346 ± 7 kg) of the same treatment (Table 6).

At 19 months of age, we finished the experiment by inducting heifers to ovulation with the insertion of an intravaginal progesterone device, which induced a CL in around 70% of heifers (Table 6). The heifers of the HH and CH treatments presented the highest BW ($P = 0.0019$) compared to restricted heifers in phase II.

Discussion

The primary aim of this study was to investigate whether a high ADG in heifers would accelerate puberty in Nellore heifers. Results showed that a high growth rate from the 3rd to 7th month of age did not increase the percentage of pubertal Nellore heifers at 19 months old. However, the study revealed that high ADG during younger age makes heifers more efficient, as demonstrated by the similarity of puberty curves between HC and CH treatments. This was unexpected

Table 4 Performance of pubertal Nellore heifers during the experiment

Variables	Treatments				*P*-value		
	HH	HC	CH	CC	F1	F2	F1*F2
BW at puberty (kg)	345 ± 11	325 ± 12	333 ± 17	346 ± 20	0.78	0.80	0.30
Age at puberty (months)	14.5 ± 0.4	16.0 ± 0.5	15.6 ± 0.5	17.6 ± 0.7	0.02	0.01	0.64
Total DMI (kg)	1,992 ± 75	1,440 ± 85	1,758 ± 78	1,360 ± 75	0.13	<.01	0.59
Puberty (%)	84 (11/13)	60 (6/10)	50 (6/12)	23 (3/13)			

Abbreviations: *HH*, heifers submitted to high average daily gain (ADG; dry matter intake (DMI) *ad libitum*) in phases I (from 3rd to 7th month of age) and II; *HC*, high ADG in phase I and control (50% DMI restricted) ADG in phase II (from 7th month of age to puberty); *CH*, control ADG in phase I and high ADG in phase II; *CC*, control ADG in the phases I and II; *BW*, body weight; *Total DMI*, dry matter intake of pubertal heifers from to 3rd month of age to puberty. *F1*, the effect of phase I, *F2*, the effect of phase II; *F1*F2*, an interaction effect between F1 and F2. The means are reported with the standard error of the mean

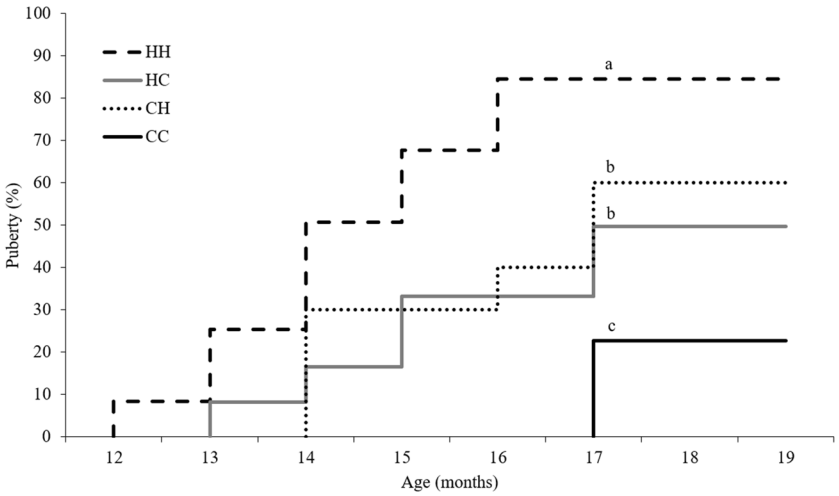

Fig. 1 The cumulative proportion (%) of Nellore heifers attained puberty in each treatment until the 19th month of age. (a–b) Lines with different lattes were statically different ($P = 0.0145$). Heifers aged 12 months in December. Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19th month of age); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, control ADG in the phases I and II

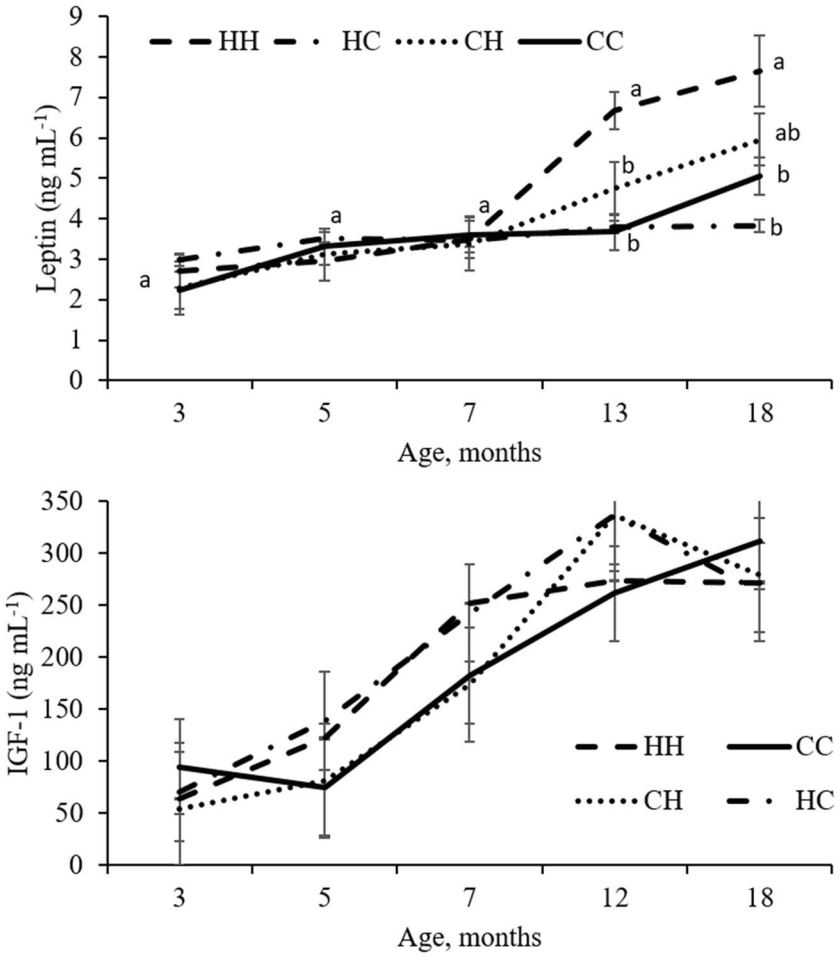

Fig. 2 The serum concentration of insulin growth factor-1 (IGF1) and leptin according to treatments at 3, 5, 7, 13, and 18 months of age. There was an interaction of leptin concentration among phases and age ($P = 0.0026$), with was demonstrated by different letters. There was no interaction in IGF1 concentration between treatments and age ($P = 0.6829$), but heifers submitted to high average daily gain (ADG) during phase I had higher ($P = 0.0220$) IGF1 concentration than control at 5 and 7 months of age. Abbreviations: HH, heifers submitted to high average daily gain (ADG; dry matter intake (DMI) *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19th month of age); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, control ADG in the phases I and II

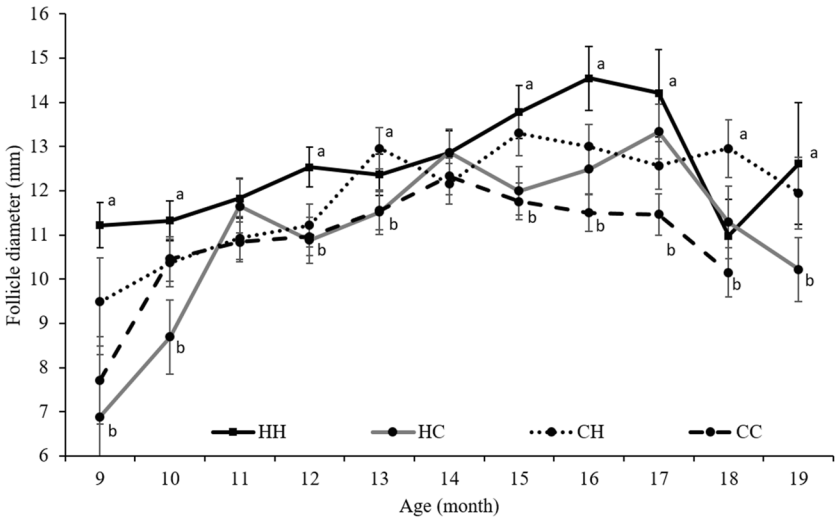

Fig. 3 Effect of treatment on the diameter of the largest follicle in prepubertal Nellore heifers. Ovaries of heifers were scanned every 2 days for 15 days once a month to determine the maximum diameter of the follicle. There was an interaction between factors and age ($P = 0.0221$). (a–b) Lines with deferment lattes were statically different ($P < 0.05$). Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th month of age to puberty); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, control ADG in the phases I and II

since HC heifers had dietary restriction during phase II for 11 months. Nonetheless, both HC and CH heifers exhibited similar cumulative puberty proportions, with CH heifers weighing more at the end of the trial but not reaching puberty. These findings have significant implications for farmers in terms of reducing costs by inducing puberty in heifers using less feed.

Our data did not corroborate the results reported by Moriel et al. (2014), who observed an accelerated puberty in Brahman × British crossbred heifers that were early weaned and submitted to a grain-based diet for 180 days. Moreover, Nellore heifers from the current trial showed smaller growth performance compared to Angus or crossbreed heifers from Gasser et al. (2006a), Cardoso et al. (2014), and Moriel et al. (2014) studies. Nellore heifers in the current study had an ADG of 0.8 kg/day and DMI of 3% of BW. In previous studies that showed the effect of metabolic imprinting before 7 months of age with the further earlier puberty, heifers had ADG greater than 1 kg/day (Gasser et al. 2006a; Cardoso et al. 2014), consuming 3% (Gasser et al. 2006a) to 3.5% (Moriel et al. 2014) of BW. Therefore, the smaller ADG observed Nellore heifers in the current trial might explain the lack of metabolic imprinting in our heifers.

During phase II, CH heifers had a higher ADG of nearly 0.1 kg/day compared to HH heifers, indicating a change in the growth curve. Dry matter intake was also approximately 0.5 kg/day lower in CH heifers than in HH heifers. This finding is consistent with the results of previous studies by Miszura et al. (2021) and Cardoso et al. (2014), which found that changes in the growth curve did not affect the age at puberty. Interestingly, HH heifers exhibited a 30% higher puberty rate than CH heifers, but the effect of BW gain on the age at puberty remains unclear in Nellore heifers.

The percentage of pubertal heifers in HH treatment (84%) until 18 months of age may be considered a paradigm break in the Nellore breed. As far as we know, no studies have shown this high percentage of spontaneous *Bos indicus* pubertal heifers at this age. Notably, there was a great genetic effect in our experiment as all heifers were daughters of the same sire that may be considered precocious for the breed standards (the best 1% for scrotal circumference in the Gensys (2019) catalog). Previous studies have indicated that genetics was more important than nutrition for puberty in *Bos indicus* heifers (Ferraz Junior et al. 2018). The current study observed that high ADG associated with early weaning in heifers with genetics favorable to sexual precocity was a tool to induce a greater percentage of puberty in heifers before their first breeding season.

The high ADG induced greater serum leptin concentration in HH from the 13th to 18th month of age, indicating that HH treatment could change the body composition of heifers, which increases fat percentage in heifers. Probably, greater serum leptin concentration was determinant in inducing a greater percentage of puberty in this treatment. However, Ferraz Junior et al. (2018) did not observe differences in serum leptin concentration in precocious and non-precocious Nellore heifers. Previous studies have shown that leptin infusion in the brain in fasting mice stimulated GnRH and LH secretion (Watanobe 2002). However, experiments with primates and rodents did not confirm this direct action

Table 5 Luteinizing hormone (LH) profile of Nellore heifers submitted to high (*ad libitum* intake) or control (50% restricted intake) average daily gain (ADG) on phase I (from 3rd to 7th month of age) and phase II (from 7th to 19th month of age)

	Maximum LH concentration (ng/mL)								The amplitude of LH pulse (ng/mL)							
Age (months)	Phase I		SEM	*P*-value	Phase II		SEM	*P*-value	Phase I		SEM	*P*-value	Phase II		SEM	*P*-value
	High	Control			High	Control			High	Control			High	Control		
7	2.66	1.97	0.70	0.11	2.39	2.25	0.48	0.81	1.87	1.20	0.68	0.52	1.63	1.40	0.52	0.70
9	4.42	3.52	0.38	0.06	4.09	3.72	0.44	0.74	3.32	2.65	0.37	0.13	3.12	2.75	0.42	0.85
11	3.20	2.53	0.40	0.22	2.68	3.30	0.41	0.27	2.21	1.70	0.39	0.43	1.75	2.38	0.40	0.33
13	3.29	3.51	0.32	0.45	2.97	3.75	0.32	0.12	2.20	2.53	0.31	0.29	1.89	2.77	0.32	0.08
15	3.93	2.86	0.32	0.15	2.86	3.37	0.32	0.54	2.72	1.85	0.31	0.15	1.87	2.25	0.31	0.09
16	3.24	2.61	0.32	0.76	2.57	2.92	0.34	0.57	2.29	1.52	0.31	0.63	1.46	1.90	0.33	0.53
17	2.97	3.24	0.33	0.41	2.52	3.56	0.33	0.09	1.90	2.09	0.33	0.39	1.34	2.47	0.33	0.07
18	2.97	3.80	0.43	0.94	3.60	3.44	0.38	0.78	1.87	2.74	0.42	0.89	2.71	2.31	0.37	0.61
19	2.05	2.99	0.57	0.84	3.44	2.23	0.74	0.30	1.08	1.82	0.55	0.25	2.26	1.13	0.71	0.12
	Mean of LH concentration (ng/mL)								Frequency of LH pulses/12 h							
Age (months)	Phase I		SEM	*P*-value	Phase II		SEM	*P*-value	Phase I		SEM	*P*-value	Phase II		SEM	*P*-value
	High	Control			High	Control			High	Control			High	Control		
7	1.08	1.01	0.18	0.35	1.07	1.03	0.10	0.75	2.00	1.60	0.58	0.32	2.00	1.50	0.48	0.06
9	1.11	1.06	0.11	0.75	1.01	1.15	0.10	0.34	1.11	1.30	0.32	0.35	1.09	1.38	0.29	0.69
11	1.20	0.94	0.11	0.08	1.04	1.10	0.10	0.70	1.67	1.10	0.32	0.42	1.45	1.25	0.29	0.39
13	1.31	1.21	0.10	0.53	1.23	1.29	0.11	0.69	1.80	1.82	0.31	0.91	1.55	2.10	0.30	0.28
15	1.34	1.30	0.15	0.83	1.20	1.44	0.10	0.21	1.67	2.33	0.47	0.02	1.43	2.63	0.45	0.01
16	1.01	1.15	0.15	0.44	1.06	1.10	0.10	0.84	1.60	2.22	0.48	0.02	1.67	2.25	0.46	0.01
17	1.36	1.33	0.15	0.88	1.31	1.38	0.11	0.69	2.20	2.71	0.47	0.71	3.00	2.25	0.48	0.25
18	1.29	1.27	0.16	0.91	1.22	1.34	0.12	0.54	2.33	1.83	0.51	0.42	1.00	2.80	0.48	0.02
19	1.13	1.12	0.16	0.95	1.23	1.02	0.12	0.29	0.33	0.83	0.51	0.69	0.75	0.60	0.48	0.64

There was no interaction among experimental phases and age in any variable ($P > 0.05$). The treatments were arranged in 2 × 2 factorial, whose first factor was high (H; 0.79 kg/day) or control (C; 0.45 kg/day) ADG from the 3rd to 7th month of age (growth phase I). The second factor was also high (H; 0.7 kg/day) or control (C; 0.5 kg/day) ADG from the 7th month of age to puberty (growth phase II). The means are reported with the standard error of the mean

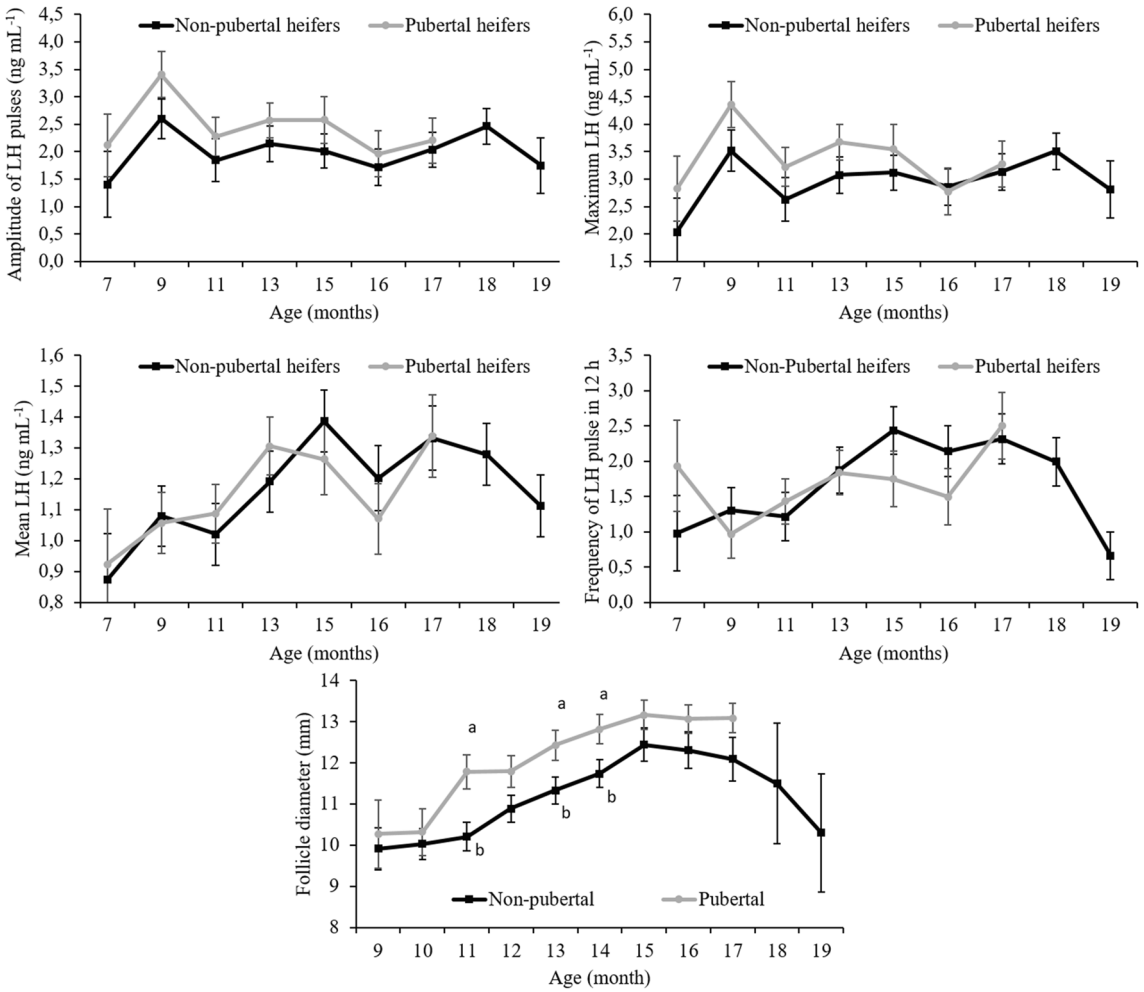

Fig. 4 Retrospective analysis of luteinizing hormone (LH) profile and the largest follicle diameter of heifers that reached puberty before 18 months of age with that of heifers that did not reach puberty. All LH profiles were collected when the heifers were not yet pubertal. Blood samples were taken before puberty, and after the first ovulation, heifers were removed from the experiment. Ten heifers that were selected for LH blood collection reached puberty. There was no effect ($P > 0.05$) of puberty status and no interactions between puberty status and age in any LH profile variable. However, at 11, 13, and 15 months, pubertal heifers had larger follicles ($P < 0.05$) than non-pubertal heifers

of leptin on GnRH-secreting neurons (Roa et al. 2010). Leptin can inhibit NPY expression (Gamba et al. 2006), modulate the expression of kisspeptin receptors (Stephens et al. 2015), and then hasten puberty. However, although chronic administration of recombinant leptin increased leptin concentration, it did not increase the frequency of LH pulses nor anticipated puberty in beef heifers (Maciel et al. 2004; Carvalho et al. 2013). Therefore, it is assumed that leptin is not responsible for puberty triggers, but acts as a permissive signal for puberty (Maciel et al. 2004; Barb et al. 2004).

The high ADG from the 3rd to 7th month of age increased serum IGF1 concentration at 5 and 7 months. A greater amount of grain in the diet increases insulin concentration, stimulating IGF1 production through an increase in the hepatic expression of growth hormone receptors (GHR-1A) (Butler et al. 2003). However, a high ADG at phase II was unable to increase serum IGF1 concentration compared with control heifers, likely to the smaller difference in ADG at phase II compared with I (0.170 and 0.340 kg/day, respectively). Heifers submitted to *ad libitum* intake (HH, CH, and HC treatments) had a serum IGF1 peak at 12 months old, just before heifers reaching puberty. Notably, a serum IGF1 increase has been associated with puberty onset (Cooke et al. 2013; Johnston et al. 2014; Ferraz Junior et al. 2018), but greater serum IGF1 was not able to induce all heifers to puberty. Therefore, serum IGF1, similar to serum leptin, is also not responsible for puberty triggers but acts as a permissive signal for puberty. Taking together leptin and IGF1 data, greater serum leptin concentration was more associated

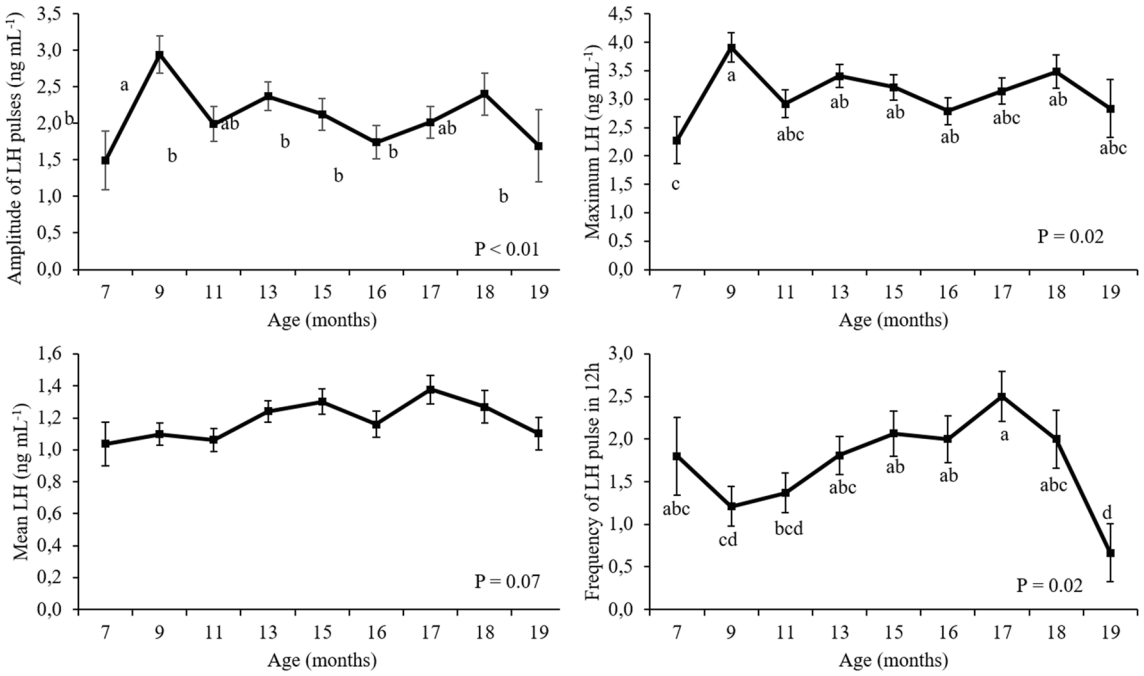

Fig. 5 Effect of age in luteinizing hormone (LH) profile in Nellore heifers. (a–b) Lines with deferment lattes were statically different ($P < 0.05$)

with greater BW. On the other hand, greater serum IGF1 was more associated with increasing DMI.

Different BW induced small effects on LH profile, likely due to the smaller difference in ADG among treatments. In Angus heifers, it was demonstrated that a decline in negative feedback exerted by estrogen in GnRH was necessary to increase the frequency of LH pulses during the prepubertal phase (Day and Anderson 1998). In the current trial, the main factor affecting LH profile was age, whose frequency of LH pulses slowly increased from the 9th to 17th month of age, but it occurred in both pubertal and non-pubertal heifers. This result disagrees with studies with Angus heifers that showed an increase in the frequency of LH pulse before puberty (Day and Anderson 1998; Gasser et al. 2006d), which normally is used as a good predictor of puberty. However, Rodrigues et al. (2002) reported that the frequency of LH pulse increased before puberty in *Bos taurus* heifers but not in *Bos indicus* heifers. Little is known about the LH profile in Nellore heifers before reaching puberty. We suggest that the first ovulation in puberty Nellore heifers occurred, beyond the LH frequency increase, due to follicular mechanisms that allowed the dominant follicle to get ovulation capacity from an increase of LH receptor and then ovulated with 2 to 2.5 LH pulses/12 h. This idea was corroborated by the greater largest follicle diameter from the 11th to 14th month of age in pubertal heifers despite a similar LH profile in puberty heifers. However, non-pubertal heifers also showed a similar increased frequency of LH pulse, which was corroborated with the similar diameter of the largest follicle since the 15th month of age. However, the current study was not able to explain why non-puberty heifers had a similar increase in the frequency of LH pulse and the largest follicle diameter, as well as leptin and IGF1 concentration.

From the 17th to 19th month of age, there was a drop in the frequency of LH pulse and the largest follicle diameter, and none of the heifers reached puberty at this age, even with all heifers having greater BW and an ascendant nutritional plan. In June, the winter solstice occurs in the southern hemisphere. Thus, we speculate that heifers had a photoperiod effect, in which short days could block puberty onset. In the same location, no heifers reached puberty from May to August for 2 consecutive years (Ferraz Junior et al. 2018). However, photoperiod in heifers is poorly studied.

The induction of puberty with progesterone has grown in Brazilian livestock (Claro Júnior et al. 2010; Rodrigues et al. 2014). At 24 months of age and about 330 kg of body weight, it is possible to induce puberty in about 90% of heifers (Rodrigues et al. 2014). In the present study, the insertion of a source of progesterone was able to induce ovulation in 70% of non-pubertal Nellore heifers at 19 months of age, regardless of body weight that ranged from

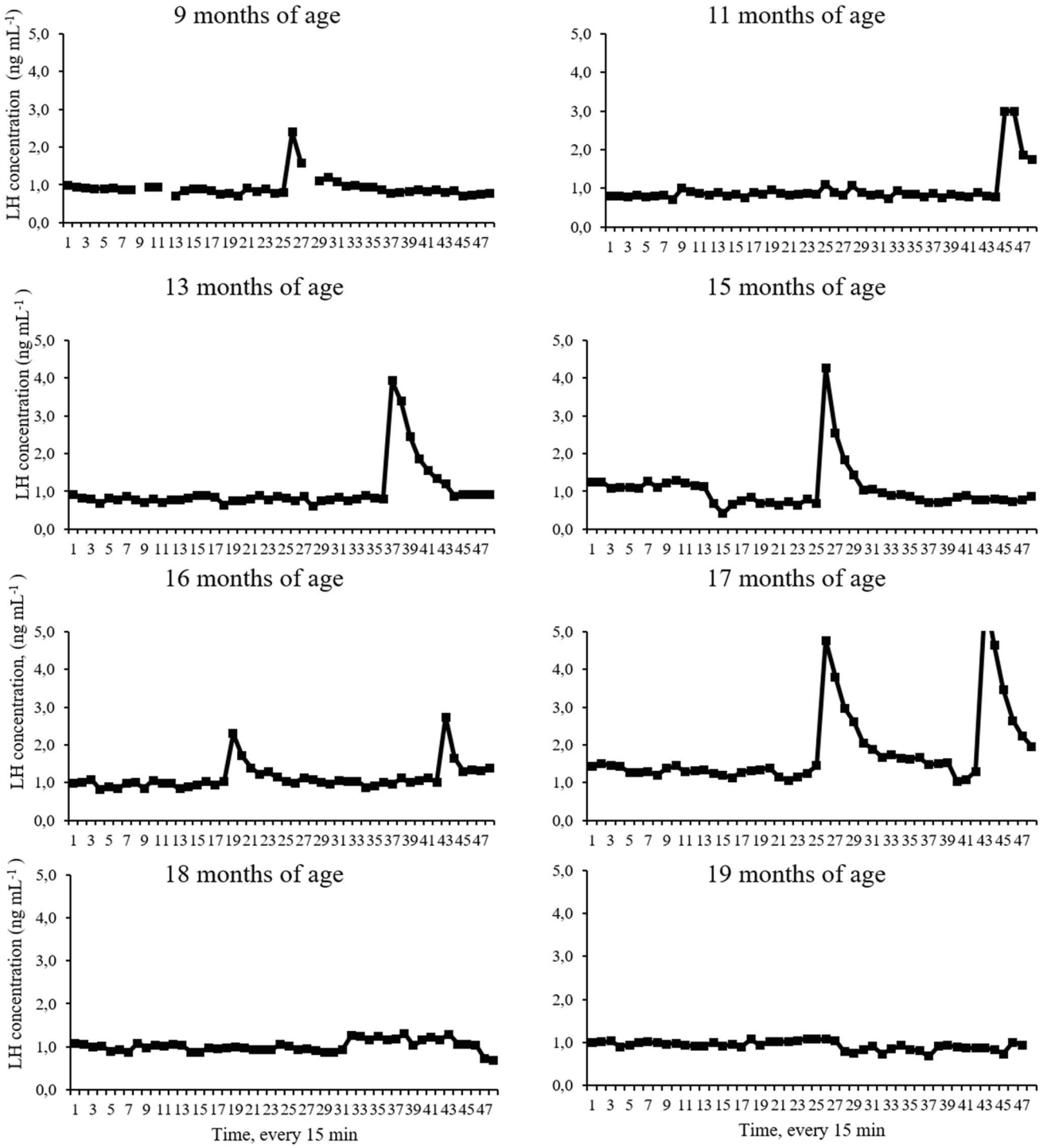

Fig. 6 Luteinizing hormone (LH) profile of a non-pubertal Nellore heifer with a body weight of 179 kg and 390 kg at the 7th and 19th month of age, respectively. Serum samples were collected every 15-min intervals for 12 h—heifer aged 12 months in December

330 to 401 kg. Progesterone is effective in inducing puberty in Charolais and Hereford heifers from 12.5 months old or greater (Hall et al. 1997). The mechanism by which progesterone induces puberty is not yet well known. Still, it is known that kisspeptin neurons are sensitive to progesterone, and progesterone receptors are needed to induce a normal LH peak via kisspeptin Hall et al. (1997).

In conclusion, high ADG was associated with earlier puberty, mainly with high ADG at younger age. The programmed fed applied in the current study increased serum

Table 6 Response to puberty induction using an intravaginal progesterone device by 9 days in non-pubertal Nellore heifers at 19th month of age

	Treatments			
	HH	HC	CH	CC
Number	2	4	6	10
Heifers with CL (%)	100 (2/2)	75 (3/4)	67 (4/6)	70 (7/10)
BW at 19 months of age (kg)	$399^{a} \pm 20$	$340^{b} \pm 14$	$401^{a} \pm 14$	$330^{b} \pm 9$

Abbreviations: *HH*, heifers submitted to high average daily gain (ADG; dry matter intake (DMI) *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19 month of age); *HC*, high ADG in phase I and control (50% DMI restricted) ADG in phase II; *CH*, control ADG in phase I and high ADG in phase II; *CC*, Control ADG in the phases I and II; *CL*, corpus luteum; *BW*, body weight

Different letters indicate statistically significant differences among treatments ($P = 0.0019$). The means are reported with the standard error of the mean

leptin in HH heifers after receiving a grain-based diet. On the other hand, serum IGF1 was more affected by changes in DMI. In addition, this study showed that LH profile was mainly affected by age.

Author contribution M.V.C. Ferraz**: data curation, investigation, writing—original draft;** M.H. Santos: **investigation;**. G.B. Oliveira: investigation. J.P.R. Barroso: investigation. D.M. Polizel: **investigation**; G.P. Nogueira: **conceptualization, methodology;** V.N. Gouvea: **conceptualization, methodology;** P.H.V. Carvalho: **writing—review and editing; J.S. Biava: investigation, writing—original draft;** E.M. Ferreira: **writing—original draft; A.V. Pires: methodology, conceptualization, funding acquisition, supervision. All authors read and approved the manuscript.**

Funding This study was financial supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014/14136-4; 16/17598-4; 18/06242-0) **and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for on this project.**

Data Availability The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval The Animal Care and Use Committee from the University of São Paulo approved all procedures with animals.

References

AOAC, editor. Official methods of analysis. 15th ed. Gaithersburg: AOAC Int; 1990.

Barb, C.R., and Kraeling, R.R., 2004. Role of leptin in the regulation of gonadotropin secretion in farm animals. Animal Reproduction Science, 82-83, 155–167.

Bolt, D.J., and Rollins, R., 1983. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone Journal of Animal Science, 56, 146-154.

Bolt, D.J., Scott, V., and Kiracofe, G.H., 1990. Plasma LH and FSH after estradiol. Norgestomet and Gn-RH treatment in ovariectomized beef heifers. Animal Reproduction Science, 23, 263-271.

Butler, S.T., Marr, A.L., Pelton, S.H., Radcliff, R.P., Lucy, M.C., and Butler, W.R., 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. Journal of Endocrinology, 176, 205–217.

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

Carvalho, M.V., Diniz-Magalhães, J., Pereira, A.S., Santos, M.V., Silva, L.F., 2013. Effect of chronic infusion of leptin and nutrition on sexual maturation of zebu heifers. Journal of Animal Science, 91, 1207-1215.

Claro Júnior, I., Sá Filho, O.G., Peres, R.F.G., Aono, F.H.S., Day, M.L., Vasconcelos, J.L.M., 2010. Reproductive performance of prepubertal Bos indicus heifers after progesterone-based treatments. Theriogenology, 74, 903–911.

Cooke, R.F., and Arthington, J.D., 2009. Plasma progesterone concentrations as puberty criteria for Brahman-crossbred heifers. Livestock Science, 123, 101–105.

Cooke, R.F., Bohnert, D.W., Francisco, C.L., Marques, R.S., Mueller, C.J., Keisler, D.H., 2013. Effects of bovine somatotropin administration on growth, physiological, and reproductive responses of replacement beef heifers. Journal of Animal Science, 91, 2894-2901.

Day, M.L., and Anderson, L.H., 1998. Current concepts on the control of puberty in cattle. Journal of Animal Science, 76, 1-15.

Ferraz Junior, M.V.C., Pires, A.V., Santos, M.H., Silva, R.G., Oliveira, G.B., Polizel, D.M., Biehl, M.V., Sartori, R., and Nogueira, G.P., 2018. A combination of nutrition and genetics is able to reduce age at puberty in Nelore heifers to below 18 months. Animal, 12, 569-574.

Gamba, M., and Pralong, F.P., 2006. Control of GnRH neuronal activity by metabolic factors: the role of leptin and insulin. Molecular and Cellular Endocrinology, 254–255, 133–139.

Gasser, C.L., Behlke, E.J., Grum, D.E., and Day, M.L., 2006a. Effect of timing of feeding high concentrate diet on growth and attainment of puberty in early-weaned heifers. Journal of Animal Science, 84, 3118-3122.

Gasser, C.L., Brindges, G.A., Mussard, M.L., Grum, D.E., Kinder, J.E., Day, M.L., 2006c. Induction of precocious puberty in heifers III: hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. Journal of Animal Science, 84:2050-2056.

Gasser, C.L., Burke, C.R., Mussard, M.L., Behlke, E.J., Grum, D.E., Kinder, J.E., and Day, M.L., 2006b. Induction of precocious puberty in heifers II: advantage ovarian follicular development. Journal of Animal Science, 84, 2042-2049.

Gasser, C.L., Grum, D.E., Mussard, M.L., Fluharty, F.L., Kinder, J.E., Day, M.L., 2006d. Induction of precocious puberty in heifers I: enhanced secretion of luteinizing hormone. Journal of Animal Science, 84, 2035-2041.

Gensys, 2013. Sumário de touros aliança Nelore 2013. Retrieved on 1 June 2013 from http://www.gensys.com.br/home/show_page.php?id=701

Gensys, 2019. Sumário de touros aliança Nelore 2019. Retrieved on 1 May 2019 from https://gensys.com.br/sumario/sumario-alianca-nelore-2019-2020/

Goodman, R.L., Karsch, F.J., 1980. Pulsatile secretion of luteinizing hormone: differential suppression by ovarian steroids. Endocrinology, 107, 1286-1290.

Hall, J.B., Staigmiller, R.B., Short, R.E., Bellows, R.A., Macneil, M.D., and Bellows, S.E., 1997. Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers. Journal of Animal Science, 75, 1606–1611.

Johnston, D.J., Barwick, S.A., Corbet, N.J., Fordyce, G., Holroyd, R.G., Williams, P.J., and Burrow, H.M., 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Animal Production Science, 49, 399–412.

Johnston, D.J., Corbet, N.J., Barwick, S.A., Wolcott, M.L., and Holroyd, R.G., 2014. Genetic correlations of young bull reproductive traits and heifer puberty traits with female reproductive performance in two tropical beef genotypes in northern Australia. Animal Production Science, 54, 74–84.

Maciel, M.N., Zieba, D.A., Amstalden, M., Keisler, D.H., Neves, J.P., and Williams, G.L., 2004. Chronic administration of recombinant ovine leptin in growing beef heifers: effects on secretion of LH, metabolic hormones, and timing of puberty. Journal of Animal Science, 82, 2930–2936.

Miszura, A.A., Ferraz, M.V.C., Cardoso, R.C., Polizel, D.M., Oliveira, G.B., Barroso, J.P.R., Gobato, L.G.M., Nogueira, G.P., Biava, J.S., Ferreira, E.M., and Pires, A.V., 2021. Implications of growth rates and compensatory growth on puberty attainment in Nellore heifers. Domestic Animal Endocrinology, 74, 106526.

Moriel, P., Johnson, S.E., Vendramini, J.M.B., Mercadante, V.R.G., Hersom, M.J., Arthington, J.D., 2014. Effects of calf weaning age and subsequent management system on growth and reproductive performance of beef heifers. Journal of Animal Science, 92, 3096–3107.

National Research Council (NRC). Nutrient requirements of beef cattle, 7th revised edition. Washington, DC: National Academic Press, 1996.

Nepomuceno, D.D., Pires, A.V., Ferraz Jr, M.V.C., Biehl, M.V., Gonçalves, J.R., Moreira, E.M., and Day, M.L., 2017. Effect of prepartum dam supplementation, creep-feeding and post-weaning feedlot on age at puberty in Nellore heifers. Livestock Science, 195, 58-62.

Ren, M.Q., Wegner, J., Bellmann, O., Brockmann, G.A., Schneider, F., Teuscher, F., Ender, K., 2002. Comparing mRNA levels of genes encoding leptin, leptin receptor, and lipoprotein lipase between dairy and beef cattle. Domestic Animal Endocrinology, 23, 371 – 381.

Roa, J., Galiano, D.G., Castellano, J.M., Pinilla, F.G.L., and Tena-Sempere, M., 2010. Metabolic control of puberty onset: new players, new mechanisms. Molecular and Cellular Endocrinology, 324, 87–94.

Rodrigues, A.D.P., Peres, R.F.G., Lemes, A.P., Martins, T., Pereira, M.H.C., Carvalho, E.R., Day, M.L., and Vasconcelos, J.L.M., 2014. Effect of interval from induction of puberty to initiation of a timed AI protocol on pregnancy rate in Nellore heifers Theriogenology, 82, 760–766.

Rodrigues, H.D., Kinder, J.E., and Fitzpatrick, L.A., 2002. Estradiol regulation of luteinizing hormone secretion in heifers of two breed types that reach puberty at different ages. Biology of Reproduction, 66, 603–609.

Stephens, S.B., Tolson, K.P., Rouse Junior, M.L., Poling, M.C., Hashimoto-Partyka, M.K., Mellon, P.L., and Kauffman, A.S., 2015. Absent progesterone signaling in kisspeptin neurons disrupts the LH surge and impairs fertility in female mice. Endocrinology, 156, 3091-3097.

Van Soest, P.J., Robertson, J.B., and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Watanobe, H., 2002. Leptin directly acts within the hypothalamus to stimulate gonadotropin-releasing hormone secretion in vivo in rats. The Journal of Physiology, 545, 255–268.