

A combination of nutrition and genetics is able to reduce age at puberty in Nelore heifers to below 18 months

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Nelore heifers usually begin their reproductive life at ≥ 24 months of age mainly due to suboptimal nutritional conditions and genetics. This study aimed to determine the effect of expected progeny difference (EPD) for age at first calving and average daily gain (ADG) on puberty in Nelore (*Bos taurus indicus*) heifers. A total of 58 weaned heifers (initial BW = 174 ± 6 kg; age = 9 ± 1 months) were allocated into 28 feedlot pens. Heifers were born from four sires, of which two had low EPD for age at first calving (L; $n = 33$) and two had high EPD for age at first calving (H; $n = 25$). Then, heifers of each EPD were randomly assigned to high ADG (HG; 0.7 kg) or low ADG (LG; 0.3 kg), resulting in four treatments: heifers from L sires were submitted to either HG (LHG; $n = 17$) or LG (LLG; $n = 16$), and heifers from H sires were submitted to either HG (HHG; $n = 12$), or LG (HLG; $n = 13$). The HG heifers were fed a 75% grain diet, whereas the LG heifers received 93% of forage in their diet. Blood samples were collected at 9, 14, 18, 24 and 28 months of age for IGF1 and leptin determination. There was a treatment effect ($P < 0.01$) on the proportion of heifers that attained puberty by 18 (62%, 0%, 0% and 0%), 24 (100%, 6%, 54% and 0%) or 36 (100%, 100%, 100% and 38%) months of age for LHG, LLG, HHG and HLG treatments, respectively. In addition, mean age at puberty was different across treatments ($P < 0.01$). Heifers from the LHG achieved puberty at the earliest age when compared with cohorts from other treatments (18.1, 28.9, 23.9 and 34.5 months for LHG, LLG, HHG and HLG, respectively). Serum IGF1 concentrations were higher for L heifers compared with H cohorts at 9, 14, 18, 24 and 28 months of age ($P < 0.01$; treatment $\times$ age interaction), whereas circulating leptin concentrations were higher ($P < 0.01$; age effect) as heifers became older, regardless of the treatments. In conclusion, only Nelore heifers with favorable genetic merit for age at first calving were able to attain puberty by 18 months of age. In heifers with unfavorable genetic merit for age at first calving, supplementary feeding to achieve high ADG was unable to shift the age at puberty below 24 months.

Keywords: age at first calving, *Bos taurus indicus*, IGF1, leptin, nutrition

Implications

Early attainment of puberty predicts anticipation of the heifers' productive life. This manuscript shows that by using semen from sires with favorable genetic merit for age at first calving and adequate nutrition, a significant percentage of heifers can attain puberty by the first breeding season. On the other hand, heifers born from sires with unfavorable genetic merit for age at first calving had delayed reproductive life even under adequate nutritional conditions. These results clearly show the importance of choosing the sire and its impact on production indexes.

Introduction

Puberty is defined as a sequence of events that results in the first ovulation and subsequently corpus luteum formation, allowing the first pregnancy of heifers (Moran *et al.*, 1989). Therefore, puberty may be defined as the beginning of the female's productive life, which was demonstrated by Brumatti *et al.* (2011) who observed that puberty onset was an important economic index in beef cattle. Reducing age at first calving from 3 to 2 years increased profitability of the cow-calf operations due to a 138 kg increase of calf produced per cow during a 12-year productive life period (Nunez-Dominguez *et al.*, 1991). In the same line, Terakado *et al.* (2015) found that Nelore heifers exposed to an early breeding season (<17 months of age) increased the calves'

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weights at weaning, and the odds for precocious heifers to remain in the herd until 5 or 6 years of age.

Nelore is the major breed beef cattle in Brazil and the heifers usually reach puberty from 2 to 3 years of age, weighing ~300 kg (Gunki *et al.*, 2001; Sereno *et al.*, 2001; Nepomuceno *et al.*, 2017). According to the Brazilian Association of Zebu Breeders, the average age at first calving in Nelore is 38 ± 5 months (ABCZ, 2016). Nonetheless, the beginning of heifers' reproductive life at 14 months of age occurs in a few production systems, when nutritional (Nepomuceno *et al.*, 2017) and genetic (Eler *et al.*, 2002) strategies are explored. Firstly, we hypothesized that high average daily gain (ADG; HG; 0.7 kg/day) in heifers born from sires with low expected progeny difference (EPD) for age at first calving (L) would contribute to reach puberty by 18 months of age, whereas HG by itself would not be sufficient to induce puberty in heifers born from sires with high EPD to age at first calving (H). Our second hypothesis was that heifers submitted to low ADG (LG; 0.3 kg/day) would reach puberty at the same age, regardless whether they were born from L or H sires. Thus, this study aimed to determine the effect of sires' EPD for age at first calving and ADG on puberty in Nelore heifers.

Material and methods

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

Animal procedure and nutritional models

A total of 58 Nelore heifers born from four sires (two sires ($n = 12$ and 13 , for sire 1 and 2, respectively) with low (EPD of sire 1 within the 10% percentile, and sire 2 within the 20% percentile) EPD for age at first calving and 2 sires ($n = 11$ and 22 , for sires 3 and 4, respectively) with high (EPD of both sires within the 90% percentile) EPD for age at first calving, according to the ABCZ (2013) and GENSYS (2013) catalogs), were ranked by initial BW (174 ± 6 kg) and age (9 ± 1 months), then allocated to 28 pens. Nutritional strategies on the present study were either high ADG (HG; 0.7 kg) or low ADG (LG; 0.3 kg). The resulting treatments are hereafter designated as: heifers born from L sires submitted to HG (LHG; $n = 17$) or LG (LLG; $n = 16$), and heifers born from H sires submitted to HG (HHG; $n = 12$) or LG (HLG; $n = 13$). Heifers were also distributed among treatments according to dam because they were generated by *in vitro* embryo production. The dam ($n = 20$ donors) was not included in the statistical model due to high variability of daughters' number per dam (1 to 14), but heifers were equally distributed among treatments.

The high and low ADG were obtained by offering different diets and feed amounts. Heifers submitted to LG were fed a grass-based diet, whereas heifers in the HG were fed a grain-based diet. The amount of diet was supplied to obtain an ADG of 0.7 kg and 0.3 kg for HG and LG, respectively.

The nutritional profile of both diets is presented in Table 1. Heifers in each treatment were allocated in drylot pens ($n = 2$ or 3 heifers/pen; 3×5 m) containing feed bunks with adequate space for heifers to eat (total space = 3 m; 1.0 to 1.5 m per heifer). In addition, heifers had *ad libitum* access to a mineral supplement (BellNutri 80; Trow nutrition, Cuiabá, MT, Brazil) and water throughout the experimental period.

Heifers were weighed every week following 16-h-fast, and dry matter intake (DMI) was adjusted to the proposed ADG within each treatment. After each BW measurement and individual ADG analysis, heifers within treatments were regrouped if the ADG difference in the same pen was higher than 0.1 kg. The coastcross haylage and concentrate were weighed (Marte; AC 10k, São Paulo, Brazil) separately on a digital scale accurate to 0.1 g and fed once a day. Once a week, the orts were collected, weighed for DMI calculation, and sampled for further analyses. The DMI was evaluated on a daily basis until puberty onset, or at 36 months of age for the eight non-pubertal heifers from the HLG treatment.

Samples of the offered feed and orts were ground in a Wiley mill (Marconi, Piracicaba, Brazil) with a 1.0 mm sieve. Dry matter content was determined by drying the samples at 105°C for 24 h. Total N was determined using the Leco FP528 instrument (Leco Corporation, St. Joseph, MI, USA) according to AOAC (2006), whereas the NDF content was determined according to the procedures described by Van Soest *et al.* (1991), using α -amylase and sodium sulfite in a 2000-Ankom system (Ankom Tech. Corp., Fairport, NY, USA).

On a weekly basis, corpus luteum presence was evaluated using a transrectal ultrasound machine (DP-2200 VET; Mindray, Shenzhen, China) with a 7.5 MHz linear-array transducer. Moreover, blood samples were collected from the coccygeal vein or artery in plain tubes for progesterone quantification to confirm pubertal status. Puberty onset was defined when a corpus luteum was detected by ultrasonography and

Table 1 Ingredients and chemical composition of experimental diets

Item	Diets ¹	
	High ADG ² (0.7 kg)	Low ADG (0.3 kg)
Ingredients (% dry matter)		
Coastcross ³	25.2	93.0
Cracked corn	62.9	0
Soybean meal	11.9	0
Soybean hulls	0	7.0
Chemical composition (dry matter basis)		
Dry matter (% as fed basis)	82.1	67.5
CP (%)	15.1	11.8
NDF (%)	36.0	73.0
Metabolizable energy (Mcal/kg) ⁴	2.9	1.9

¹Mineral mix was supplied in mineral boxes *ad libitum*.

²Diet from high average daily gain (ADG) contained 30 ppm of sodic monensin (Rumensin 100, Elanco Brazil, São Paulo, SP, Brazil).

³Haylage.

⁴Estimative from NRC (1996).

confirmed by serum progesterone concentration higher than 1.5 ng/ml (Cooke and Arthington, 2009).

Hormone assays

Blood samples collected at 9, 14, 18, 24 and 28 months of age were analyzed for IGF1 and leptin concentrations. Blood samples were centrifuged for 15 min at 1800 × g and the harvested serum was frozen at –20°C until further analysis. Total IGF1 concentration was measured by a chemiluminescent assay using commercial IMMULITE 1000 kits (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and the intra-assay CV was 2.7% and 3.4% for the low and high adjuster, respectively, and the sensitivity of the assay was 20 ng/ml. Progesterone concentrations were also measured by a chemiluminescent assay using commercial IMMULITE 1000 kits (Siemens Healthcare Diagnostics). The intra-assay CV was 3.4% and 5.7% for the low and high adjuster, respectively, and the sensitivity of the assay was 0.20 ng/ml. Leptin concentrations were evaluated by a commercial RIA kit (Multi-Species Leptin; Millipore-XL-85K, Bedford, MA, USA), as reported previously (Ren *et al.*, 2002). The intra-assay CV was between 2.1% and 2.6%, whereas the inter-assay CV was between 2.4% and 9.0%, and the sensitivity of the assay was 1.0 ng/ml.

Statistical analyses

Due to lameness problems, one heifer from the HHG treatment was removed from the experiment, and not included in the statistical analysis. Heifers were used as experimental units to evaluate the effects of the treatments on BW, age at puberty and ADG until puberty because the ADG was controlled weekly. The pens were used as the experimental units to evaluate the effect of the treatments on DMI. 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), and Satterthwaite approximation was used to determine the denominator degrees of freedom for the

treatments effect. The MIXED procedure also evaluated the effect of treatments on IGF1 and leptin concentration using repeated measures over time, which were included on covariance matrices and tested for 'compound symmetry, heterogeneous compound symmetry, autoregressive, autoregressive heterogeneous, unstructured, banded, variance components, toeplitz and heterogeneous toeplitz,' and chosen according to the lowest value obtained for Akaike's information criterion. The means were obtained by the LSMEANS command and mean comparisons were performed by PDIFF option. The percentage of pubertal heifers according to each treatment at 18, 24 and 36 months of age was analyzed by the GLIMMIX procedure using the binomial option. The cumulative proportion of pubertal heifers was analyzed by logistic regression using LIFETEST procedure, and the difference of curves was assessed by the LOGRANK test.

Results

Although heifers from the HHG group were offered a diet to provide a high gain, they remained around 170 days longer in the feedlot compared with LHG cohorts, which increased ($P < 0.01$) BW (108 kg more), and decreased ($P < 0.01$) ADG at puberty (Table 2). However, HHG heifers (0.781 ± 0.03 kg) had a similar ADG as compared with LHG cohorts (0.744 ± 0.03 kg) at 18 months of age. No differences were observed on the ADG between heifers from the LG treatment ($P = 0.75$), whereas HLG heifers were heavier ($P < 0.01$) at puberty when compared with LLG cohorts (390 v. 340 kg for HLG and LLG, respectively; Table 2).

There was no treatment × period interaction ($P = 0.80$) for the circulating leptin concentrations. Regardless of the treatment, leptin concentrations were greater ($P < 0.01$) as heifers became older (Figure 1). Conversely, a treatment × age interaction ($P < 0.01$) was observed on the circulating concentrations of IGF1, in which heifers born from sires with low EPD for age at first calving had greater IGF1 concentrations than heifers born from sires with high EPD for age at

Table 2 Performance of Nelore heifers according to each treatment until 36 months of age

Item	Treatments				SE	P value
	LHG	HHG	LLG	HLG ¹		
<i>n</i>	17	11	16	13		
Initial BW (kg)	174	174	172	177	6	0.97
Puberty at 18 months (%)	62 ^a	0 ^b	0 ^b	0 ^b		<0.01
Puberty at 36 months (%)	100 ^a	100 ^a	100 ^a	38 ^b		<0.01
BW at puberty (kg)	360 ^{ab}	468 ^c	340 ^a	390 ^b	15	<0.01
Age at puberty (months)	18.1 ^a	23.9 ^b	28.9 ^c	34.5 ^d	1.1	<0.01
ADG at 18 months (kg)	0.744 ^a	0.781 ^a	0.270 ^b	0.242 ^b	0.035	<0.01
ADG at puberty (kg)	0.739 ^a	0.646 ^b	0.292 ^c	0.276 ^c	0.033	<0.01
DMI (kg/day)	5.60 ^a	5.91 ^b	4.46 ^c	4.48 ^c	0.10	<0.01

LHG = heifers born from sires with low expected progeny difference (EPD) for age at first calving submitted to high average daily gain (ADG); LLG = heifers born from sires with low EPD for age at first calving submitted to low ADG; HHG = heifers born from sires with high EPD for age at first calving submitted to high ADG; HLG = heifers born from sires with high EPD for age at first calving submitted to low ADG.

^{a,b}Values within a row with different superscripts differ at $P < 0.05$.

¹Eight heifers from the HLG treatment did not reach puberty by 36 months of age.

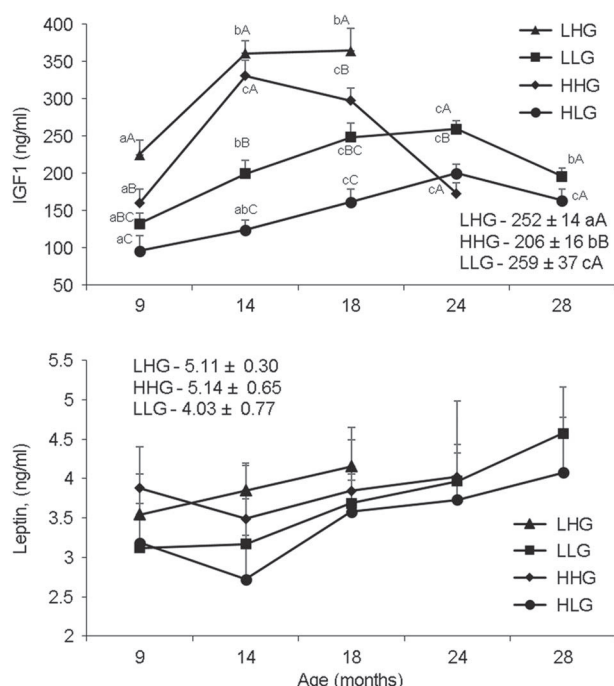


Figure 1 Serum concentration of IGF1 and leptin according to treatments at 9, 14, 24 and 28 months of age, and at the week of ovulation. There was no interaction of leptin concentration between treatments and time ($P=0.80$). Leptin concentration increased ($P<0.01$) along the age. There was interaction on IGF1 concentration between treatments and time ($P<0.01$). Treatments: heifers born from sires with low expected progeny difference (EPD) for age at first calving submitted to either high average daily gain (ADG = 0.7 kg; LHG; $n=17$) or low ADG (0.3 kg; LLG; $n=16$); heifers born from sires with high EPD for age at first calving submitted to either high ADG (HHG; $n=11$) or low ADG (HLG; $n=13$). Text box in figure shows IGF1 and leptin concentrations on the week of ovulation, which was not shown in HLG treatment because only five (5/13) heifers attained puberty. ^{A,B}Capital letters show statistical difference at $P<0.05$ within each age. ^{a,b}Small letters show statistical difference at $P<0.05$ among ages.

first calving in all ages and in the same ADG (sire effect; Figure 1). In addition, heifers submitted to greater ADG had a robust increase in plasma IGF1 concentration from 9 to 14 months of age ($P<0.01$). In contrast, heifers submitted to the low ADG had a lower increase in IGF1 concentration as age advanced ($P<0.01$; Figure 1).

There was a treatment effect ($P<0.01$) in the percentage of heifers that reached puberty by 18 (62%, 0%, 0% and 0%), 24 (100%, 6%, 54% and 0%), and 36 months of age (100%, 100%, 100% and 38%) for LHG, LLG, HHG and HLG, respectively (Table 2; Figure 2). Only five (5/13) heifers from HLG treatment reached puberty by 36 months of age, and the other 8 non-pubertal heifers weighed 362 ± 6 kg at the end of the study.

Heifers from the LHG treatment were younger ($P<0.01$) at puberty (18 ± 1 months of age) compared with the other treatments. These heifers started reaching puberty at 14 months of age, and all heifers were pubertal by 23 months of age (Table 2; Figure 2). Heifers from all treatments did not reach puberty between June to August, which coincided with the winter solstice in the South hemisphere.

Discussion

The results obtained from the LHG treatment demonstrate that it is possible to decrease the puberty age in Nelore heifers to 18 months, and it is feasible to raise Nelore heifers to ovulate before this time, matching with the end of the breeding season in tropical countries (Nepomuceno *et al.*, 2017). In order to achieve it, sires were selected for precocious age at first calving and heifers had unrestricted growth. These conditions resulted in more than half of heifers reaching puberty before the aforementioned target age of 18 months, since the LHG treatment anticipated puberty in 62% of the heifers. Previous studies with greater

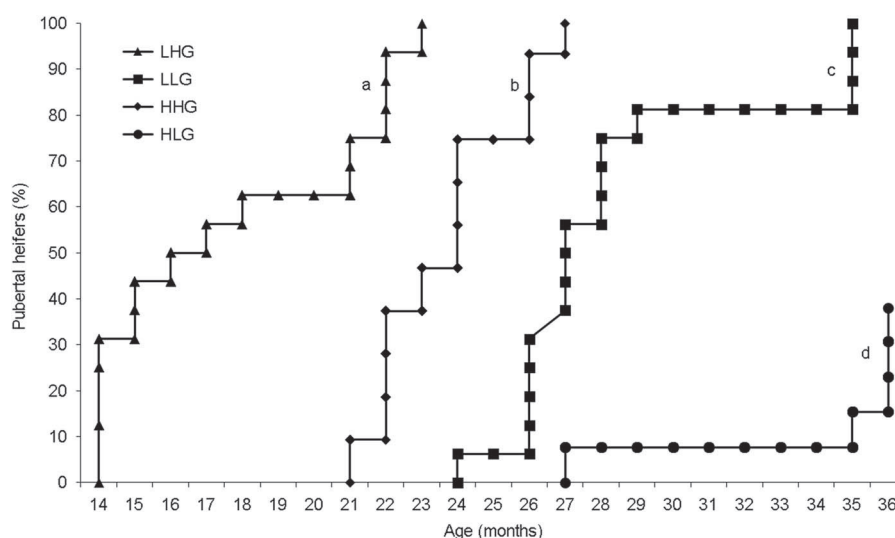


Figure 2 Cumulative proportion (%) of Nelore heifers that attained puberty in each treatment. Treatments: heifers born from sires with low expected progeny difference (EPD) for age at first calving submitted to either high average daily gain (ADG = 0.7 kg; LHG; $n=17$) or low ADG (0.3 kg; LLG; $n=16$); heifers born from sires with high EPD for age at first calving submitted to either high ADG (HHG; $n=11$) or low ADG (HLG; $n=13$). ^{a,b,c,d} Lines with different superscripts differ at $P<0.05$.

number of non-selected Nelore heifers, submitted to either feedlot or pasture systems after weaning, resulted in only 32% and 13% of puberty by 18 months of age, respectively (Nepomuceno *et al.*, 2017).

Several farmers in Brazil aim to begin the heifers' reproductive life around 26 months of age (Gunski *et al.*, 2001; ABCZ, 2016). However, it does not occur in the majority of Brazilian cow-calf systems, in which heifers are usually bred only when they are >3 years of age (Gunski *et al.*, 2001; Sereno *et al.*, 2001). Thus, the difference in the percentage of pubertal heifers between LLG and HLG treatments has immediate applicability to the beef cattle industry.

Corroborating our first hypothesis, the high ADG was not sufficient to induce puberty in heifers from sires with high EPD for age at first calving before 18 months of age. Hence, the EPD for age at first calving was essential to determine the timing of puberty onset, delaying around 5 months the age at puberty in high gain condition. Our second hypothesis was not accepted because 100% of the heifers from sires with low EPD for age at first calving reached puberty before 36 months of age, whereas only 38% of heifers from sires with high EPD for age at first calving reached puberty at the same age. Irano *et al.* (2016) suggested that the genetic control of puberty is susceptible to a pattern of complex polygenic inheritance. Several researchers attempted to identify the physiological processes that lead to this difference, and it seems that puberty onset is controlled by the hypothalamus. Cánovas *et al.* (2014) analyzed eight tissues of pre- and post-pubertal Brangus heifers by RNA-Seq and found that hypothalamus samples had the greatest increase in the puberty regulatory genes as compared with the other analyzed tissues (pituitary gland, uterus, endometrium, ovary, liver, muscle and adipose tissue). In addition, several studies with Nelore heifers have shown that single nucleotide polymorphisms provide relevant information to help elucidate which genes affect puberty onset (Costa *et al.*, 2015; Regatieri *et al.*, 2017).

The common recommendation for *Bos taurus taurus* beef heifers is to achieve 60% to 65% of their expected mature BW before the first breeding (Patterson *et al.*, 1992; Gasser, 2013), and it is believed the mature BW in Nelore is between 400 and 500 kg (Rosa *et al.*, 2000 and 2001). Rosa *et al.* (2001) found that the mature BW of cows was 447 kg after evaluating 34 herds in 11 Brazilian regions. Assuming 450 kg as the mature BW of Nelore cows, the LHG and HHG treatments resulted in BW at puberty of 80% and 107% of this mature BW, respectively. Thus, we observed that Nelore heifers in the HHG and LHG treatments reached 60% to 65% of mature BW at 14 months of age (data not shown), but unlike *B. taurus taurus* heifers, did not reach puberty at this point.

Heifers from the HHG treatment had lower ADG (0.1 kg) during the experimental period than heifers from the LHG treatment because of a longer feedlot period. This feeding length increase resulted in fatter non-precocious heifers, suggesting that they became less energetically efficient due to sigmoid type growing pattern in ruminants (NRC, 1996), corroborating the similar ADG until 18 months of age in

heifers from LHG and HHG treatments. In addition, the longer feedlot period increased the amount of feed provided for heifers from HHG treatment, increasing feed costs.

The age at puberty was also jeopardized when the heifers' development was restricted by low ADG. In our results, this difference was nearly 1 year, comparing the age at puberty in the same EPD group, showing how important a proper nutrition is. However, it is important to highlight that the probability of pregnancy at 16 months of age is not highly related to the genetic selection for weight at weaning, yearling or maturity (Bologn and Albuquerque, 2011).

The IGF1 concentration was higher in heifers from sires with low EPD for age at first calving in both ADG, indicating that IGF1 concentration was associated with genetic factors that may have contributed to an earlier puberty onset in precocious heifers. In the same line, authors have observed a correlation between IGF1 after weaning and puberty onset (Yilmaz *et al.*, 2006; Johnston *et al.*, 2014). In addition, Fortes *et al.* (2013) observed that genes in the IGF1 pathway were associated to puberty age in Brahman heifers. Chronic administration (every 14 days) of recombinant bovine somatotropin, from weaning to the beginning of the breeding season, increased IGF1 concentration and hastened puberty attainment in beef heifers (Cooke *et al.*, 2013). Besides genetic factors, IGF1 concentration was also influenced by ADG. Heifers with high ADG had a robust increase on IGF1 concentrations from 9 to 14 months of age, influenced by 75% of grain in the diet, coinciding with the growth spurt. Diets with high amount of grain increase insulin concentration, stimulating IGF1 production through an increase in the hepatic expression of growth hormone receptors (GHR-1A; Butler *et al.*, 2003). Thus, the high ADG allowed heifers to express their genetic potential. On the other hand, heifers submitted to low ADG had gradual, but slow, increase in IGF1 concentration since their diet contained 93% low-quality forage.

Leptin concentration only increased as heifers became older, demonstrating that it did not trigger puberty onset, agreeing with Maciel *et al.* (2004), who demonstrated no effect of chronic administration of recombinant ovine leptin on age at puberty in beef heifers. Nonetheless, leptin concentration has been positively associated with GnRH and gonadotropin synthesis, hence, the leptin does not trigger puberty, but it is a permissive signal for puberty onset (Maciel *et al.*, 2004; Roa *et al.*, 2010).

An interesting observation was that in June, July and August heifers from all treatments did not reach puberty, whereas in September there was a higher proportion of heifers reaching puberty. In the Southern hemisphere, September marks the beginning of the spring and hence, the day length is augmented progressively. We speculate that the lack of pubertal heifers from June to August might be related to a seasonal (photoperiod) effect, since others have already described this effect in cattle (Hansen, 1985; Schillo *et al.*, 1992). However, it is important to highlight that more studies are necessary to elucidate the mechanisms in Nelore females.

In summary, only Nelore heifers with favorable genetic merit for age at first calving were able to attain puberty by 18 months of age. In heifers with unfavorable genetic merit for age at first calving, supplementary feeding to achieve high ADG was unable to shift the age at puberty below 24 months. Moreover, the low EPD for age at first calving was an essential factor for puberty either in high or low ADG.

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