https://doi.org/10.22319/rmcp.v12i4.5796

Article

Relationships between seasonality, body characteristics and leptin at the beginning of puberty in *Bos taurus taurus* and *Bos taurus indicus* heifers in the Mexican tropics

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Abstract:

The study analyzed, in two years, the effects of breed [Brahman (BHM; n= 65); Braunvieh (BR; n= 56)], supplementation with Zilpaterol® (ZIL; treated or control), season of birth (spring or autumn) and their interactions on body surface (BS), age (APB), body weight (WPB), body condition (BC), long dorsal muscle (DM) depth, dorsal fat (DF) thickness and serum leptin concentration (LEP) at puberty (PB) of 121 heifers. At PB, BMHs were heavier and older than BRs (376.8 ± 7.4 *vs* 302.0 ± 6.6 kg; 588.1 ± 14.7 *vs* 445.5 ± 12.5 days). ZIL increased APB, WPB, BC and DM, but did not affect DF and LEP. BHMs had 18 % more BS than BRs. However, the difference in WPB/BS was only 6.4 %. When metabolic weight (MW) was used as a proportion of BS (MW/BS) instead of WPB, the difference between BHM and BR disappeared (P>0.05). The DF was 63.7 % higher in BHM than in BR. Those born in spring started PB with 24.4 % less DF than those born in autumn. Most of the BHM heifers (73.8 %) started PB in the months when light hours were increasing (P<0.05), while in BRs, the beginning of PB was uniformly distributed throughout the year, regardless of the length of light hours; this effect was present in the two years of study. It is concluded that the establishment of puberty is a multifactorial phenomenon; seasonality affects BHM and BR differently and, apparently, BS is an important factor, probably associated with efficiency in energy use. This paper reiterates the importance of dorsal fat and documents, for the first time, MW/BS and its association with the establishment of puberty.

Key words: Puberty, Body surface, Seasonality, Tropical cattle, Leptin.

Received: 04/09/2020

Accepted: 03/09/2021

Introduction

Among the most important determinants for the establishment of puberty in heifers for beef production are age[1], height and body weight[2], with marked differences between breeds. In tropical conditions, production systems with *Bos taurus taurus, Bos taurus indicus* cattle and their crosses are currently common, and the overall production and productivity of the beef production system depends on the reproductive performance of breeding herds. The prepubertal stage of heifers is a cost factor and age at first calving is an important indicator of the reproductive performance of the herd. Zebu heifers (*Bos taurus indicus*) require more age and weight to reach puberty than *Bos taurus taurus*[3]. This fact has been highlighted as a limiting factor for the reproductive success of *Bos taurus indicus* breeds(4). Age at puberty is closely related to the weight and body composition of animals(5).

There are complex interactions between different hormones for the establishment of puberty(6) and the importance of leptin is highlighted for its association with fat accumulation, since it participates in the regulation of food intake, and is a good dynamic indicator of body condition and nutritional status in ruminants(7). Circulating concentrations of leptin have been reported to increase during pubertal development in heifers(8). The available information suggests that there is a critical level of fat required for the start of reproductive activity(9) and that there may be differences in this critical fat limit between breeds(10), implying that conditions must exist to regulate the accumulation of excess energy in the form of body fat. Although several factors are known to affect the establishment of puberty in heifers, there is little literature on the interactions between these and, particularly, on the differences between *B. taurus taurus* and *B. taurus indicus*, especially if it is consider the seasonality factor, which is associated with Zebu cattle(3,11,12). Based on the above, the present study aimed to determine the association between some markers of body composition, the internal environment of animals and those of the external environment, with the establishment of puberty in *B. taurus taurus* and *B. taurus indicus* heifers, fed individually to have similar weight gains, born in different seasons of the year, with a different body composition, induced during their growth.

Material and methods

Localization

The study was carried out in the Las Margaritas experimental station, dependent on INIFAP, located in Hueytamalco, Puebla, Mexico, at 19º 51’ 03” NL and 97º 12’ 48” WL, at 500 m asl. The climate is semi-warm humid subtropical Af(c), with average annual temperature of 20.8 °C, average annual rainfall of 3,000 mm and relative humidity of 90 %.

Treatments

The study was repeated for two years; in total, 121 heifers from two seasons of birth were used: 24 Braunvieh (BR) and 33 Brahman (BHM) born in spring (May 4 ± 36 d), and 32 BR and 32 BHM born in autumn (October 27 ± 35 d). Half of the heifers of each breed and season-year received one of two treatments: 1) with the β-agonist Zilpaterol hydrochloride (ZIL, Zilpaterol®); 0.15 mg/kg from 220 to 300 kg of body weight and 0.25 mg/kg from 301 kg of body weight until the first ovulation, mixed in the concentrate, and 2) without β-agonist (control). PB for this study was defined as the first ovulation that preceded the first estrous cycle with the formation of a corpus luteum of normal duration (≥ 12 ≤ 17 d), corresponding to a regular estrous cycle (21 ± 4 d)[13].

General handling

The heifers entered the study at around seven months of age. They were housed individually in pens of 4 × 6 m, with cement floor, asbestos roof (4 × 3 m), feeder and water trough. For their adaptation to handling and sampling routine, heifers were introduced to pens approximately 30 d before the study, halter broken (2 h/day) and brushed manually. The feed consisted of free access to fresh chopped cane (*Saccharum sinense*) and commercial concentrate (18 % crude protein and 70 % TDN), in amounts adjusted individually after each weighing of the heifers to obtain similar weight gains between animals. For practical reasons, it was decided to name all females indistinctly “heifers” from the beginning until their exit from the experiment.

Estimation of development parameters and body composition

The animals were weighed every 14 days, after removal of feed and water for 18 h. Every 21 days, the body condition (1 to 9; 1= very thin, 9= obese)[14] was recorded, which was rated independently by three people, and the average of these ratings was used as a response variable. Body surface was measured every 48 days, with a counter adapted to a roller instrument to measure the surface of the trunk (odometer); in addition, a tape measure was used to determine the surface of limbs, head, ears and tail. To ensure the reproducibility of the measurements, the odometer was validated by independent measurement of 10 animals, by three people on three occasions in the same day. The intraclass correlation coefficient of the individual measures and the means were 0.96 and 0.98 (P<0.0001), respectively, and a reliability of 0.98 in Cronbach’s Alpha[15] was obtained. The area measured with the odometer was established with the circumference of the tires, the distance between them and the number of turns.

To calculate the body surface (BS), the following formula was used: BS= $2(a + l + m + e) + t + h$, where: a= area measured with the odometer, l= leg area, m= forelimb area, e= ear area, *t*= tail area and *h*= head area. The general measurements, made with tape measure, were entered into an Excel database and validated in the same way as the odometer, obtaining an intraclass correlation coefficient of 0.99 for the individual measures and the average (P<0.0001). The thickness of the dorsal fat (DF) and the depth of the long dorsal muscle (DM) were measured, obtaining images with an ultrasound (equipped with a 3.5 MHz transducer) on the left side of the back, 12 cm from the midline, at the level of the twelfth rib, after hair removal of the area and application of gel(16). The measurements were made every 14 days, coinciding with the weightings of the animals throughout the study. DM measurements correspond only to heifers of the second year of experimentation.

Blood collection and hormone measurement

At the beginning of the study, to confirm the prepubertal state of the heifers by means of serum progesterone, a blood sample was collected from each animal for five consecutive days, by puncture of the jugular vein with needle and vacuum tubes without anticoagulant. Subsequently, samples were collected four times a week until each heifer reached 230 kg of body weight. From that moment, the sampling was daily until the end of the study, which was when the beginning of puberty (PB) was determined, confirmed with the identification of the first ovulation by ultrasound. The samples were processed to obtain serum, which was frozen at -20 °C until the progesterone (P4) concentration was determined by radioimmunoassay (RIA). The P4 concentration was used as confirmation of the prepubertal state (values < 1 ng/ml) of the heifers. Serum leptin concentration (LEP) was assessed with ruminant-specific RIA(17). Serum leptin concentrations were quantified from samples taken four times a week from the start of the experiment. Once the beginning of PB was identified, the last 12 samples prior to the first ovulation were selected, from which the average value of the serum leptin concentration at puberty was obtained; the data obtained were only from the heifers of the second year of experimentation.

Ultrasonography of ovarian structures

Once the hand could be inserted through the rectum of the heifers, approximately at 230 kg and 10 mo of age, ultrasonographic images of the ovaries were taken to identify the first ovulation; initially, twice a week and then daily. For this, a Sonovet equipment was used, with a 7.5 MHz rectal transducer and a video recorder. The first ovulation was considered the first day that luteal tissue was detected preceded by the sudden disappearance of the dominant follicle, the foregoing corroborated with serum P4.

Variables

The response variables were the values at PB of: age (APB; days), body weight (WPB; kg), metabolic weight (MW; body weight elevated to the power 0.75; kg), BS (m^2), body condition (BC; points), DF (cm), DM (cm), body weight between body surface (WPB/BS; g/cm^2), metabolic weight between body surface (MW/BS; g/cm^2) and serum leptin concentration (LEP; ng/ml). The variables WPB/BS and MW/BS were generated to know how many grams of metabolically active tissue there were for each square centimeter of skin, which is an organ that participates in the regulation of body temperature and can act as a heat energy diffuser.

Statistical analyses

Data were analyzed by analysis of variance and Pearson's correlation. The design was a completely randomized one with a 2×2×2 factorial arrangement. The preliminary statistical model included the main effects of breed (BD), season of birth (SB) and treatment (with or without β-agonist), the double and triple interactions between these effects, the body weight of entry to the experiment as a covariate and, as a block, the group of entry to the experiment (animals grouped according to the date and exact year of study) nested in BD x SB. The final statistical model included the main effects and the block. In addition, for APB and BC, the model included body weight at the start of the experiment; the BD x SB interaction was only included in the definitive BC analysis. To analyze LEP, an analysis of covariance of serum leptin concentrations was performed on the days from the start of the study to the start of PB, including the fixed effects of BD, SB and treatment. The differences between means were determined with the PDIFF option, all this with the GLM procedure of SAS[18]. A homogeneity test was performed with a Chi-square to observe the effect of seasonality at the PB of the heifers, using the categorical variables BD and SB, with respect to the light hours (increasing or decreasing) at PB.

Results

Correlations between variables

Regardless of the breed of heifers, a high correlation between WPB and APB (r= 0.86; *P*<0.01) was found, as well as between BS and APB (r= 0.84; *P*<0.01). Likewise, BS was correlated with WPB and MW (r= 0.90; *P*<0.01). For LEP and WPB, a low correlation was observed (r= 0.28; *P*<0.01), as well as for LEP and BS (r= 0.37; *P*<0.01). An intermediate correlation was observed for the variables MW/BS and DF (r= 0.52; *P*<0.01). The covariate weight of the heifers at the beginning of the experiment was only significant for APB (*P*<0.01) and BC (*P*<0.05). An effect of the BD x SB interaction on BC (*P*<0.05) was found. In addition, there was a linear relationship (*P*<0.01) of LEP with respect to the days elapsed until PB, with 35 pg/ml more LEP for each day that puberty approached.

Effect of breed on variables related to puberty

At PB, BHM heifers were 74.8 kg heavier than BRs (*P*<0.0001), an age-related difference, as BHM required 142.6 d more than BRs for PB to occur (*P*<0. 0001; Table 1). BHM heifers had 0.64 m² (18 %) more BS than BRs (*P*<0.0001), while the WPB/BS ratio was 6.4 % higher (*P*<0.001) in BHMs; this difference disappeared when the comparison was made based on MW/BS. BHM heifers showed 0.4 % more (*P*<0.05) BC than BRs (7.72 *vs* 7.69 points) at PB. The most obvious difference was in DF, where BHMs showed 64 % more DF at PB than BRs (*P*<0.0001). Coinciding with the above, BHM heifers had 20 % more (*P*<0.0001) LEP at PB than BRs.

Table 1: Least squares means and standard errors for puberty response variables, for breed and treatment effects

	Breed		**Treatment**		
Variable[1]	**Brahman**	**Braunvieh**	**Control**	**Zilpaterol**	**Average**
WPB, kg	376.77 ± 7.42[a]	301.97 ± 6.59[b]	320.47 ± 6.25[a]	358.27 ± 6.42[b]	339.37 ± 6.78
MW, kg	85.34 ± 1.29[a]	72.32 ± 1.15[b]	75.57 ± 1.09[a]	82.09 ± 1.12[b]	78.83 ± 1.18
APB, days	588.13 ± 14.71[a]	445.51 ± 12.48[b]	490.29 ± 11.96[a]	543.35 ± 12.42[b]	516.82 ± 13.08
BS, m^2	4.22 ± 0.07[a]	3.58 ± 0.06[b]	3.79 ± 0.05[a]	4.00 ± 0.06[b]	3.90 ± 0.06
WPB/BS, g/cm^2	8.99 ± 0.11[a]	8.45 ± 0.10[b]	8.48 ± 0.09[a]	8.97 ± 0.10[b]	8.72 ± 0.10
MW/BS, g/cm^2	2.05 ± 0.02[a]	2.03 ± 0.02[a]	2.01 ± 0.02[a]	2.07 ± 0.02[b]	2.04 ± 0.02
BC, 1 to 9	7.72 ± 0.06[a]	7.69 ± 0.05[b]	7.56 ± 0.05[a]	7.86 ± 0.05[b]	7.71 ± 0.06
DM, cm*	5.86 ± 0.10[a]	4.69 ± 0.10[b]	4.95 ± 0.09[a]	5.60 ± 0.09[b]	5.28 ± 0.10
DF, cm	2.39 ± 0.07[a]	1.46 ± 0.06[b]	1.95 ± 0.06[a]	1.91 ± 0.06[a]	1.93 ± 0.06
LEP, ng/ml*	3.30 ± 0.10[a]	2.75 ± 0.09[b]	2.95 ± 0.09[a]	3.09 ± 0.09[a]	3.02 ± 0.09

[1]WPB=body weight; MW= metabolic weight; APB= age; BS= body surface; WPB/BS= body weight between body surface; MW/BS= metabolic weight between body surface; BC= body condition; DM= depth of the long dorsal muscle; DF= thickness of dorsal fat; LEP= serum leptin concentration. *Results of the second year.
[a,b] Means with different literal between columns of each fixed effect in each of the response variables indicate difference (*P*<0.05).

Effect of treatment on variables related to the beginning of puberty

Treatment with ZIL had a significant (*P*<0.01) effect on WPB; animals that received ZIL required 37.8 kg more body weight to begin PB. As for APB, they took 53 d longer than the untreated ones (*P*<0.05); in addition, their BS and WPB/BS were 7.0 (*P*<0.001) and 6.0 % higher (*P*<0.05), respectively. The BC and DM at PB of the animals supplemented with ZIL was 4.0 % and 13.1 % higher (*P*<0.001) than that of those in the control group, respectively. On the contrary, there was no significant (*P*>0.05) difference between the two groups with respect to DF and LEP.

Effect of the season of birth on variables related to the beginning of puberty

Heifers born in spring had 0.42 cm less DF than heifers born in autumn (*P*<0.05). For LEP, at PB, 0.46 ng/ml more was found in heifers born in autumn than in those born in spring (*P*<0.001). BS was higher in those born in spring, while WPB/BS and MW/BS were lower (*P*<0.05) in heifers born in spring (Table 2).

Table 2: Least squares means and standard errors for puberty response variables, for the season of birth effect

	Season of birth	
Variable	**Spring**	**Autumn**
WPB, kg	342.49 ± 6.55^a	336.25 ± 7.44^a
MW, kg	79.38 ± 1.14^a	78.28 ± 1.29^a
APB, days	533.19 ± 12.42^a	500.46 ± 14.51^a
BS, m^2	4.04 ± 0.06^a	3.75 ± 0.06^b
WPB/BS, g/cm^2	8.46 ± 0.10^a	8.98 ± 0.11^b
MW/BS, g/cm^2	1.97 ± 0.02^a	2.10 ± 0.02^b
BC, 1 to 9	7.68 ± 0.09^a	7.73 ± 0.06^a
DM, cm*	5.32 ± 0.10^a	5.23 ± 0.10^a
DF, cm	1.72 ± 0.06^a	2.14 ± 0.07^b
LEP, ng/ml*	2.79 ± 0.10^a	3.25 ± 0.09^b

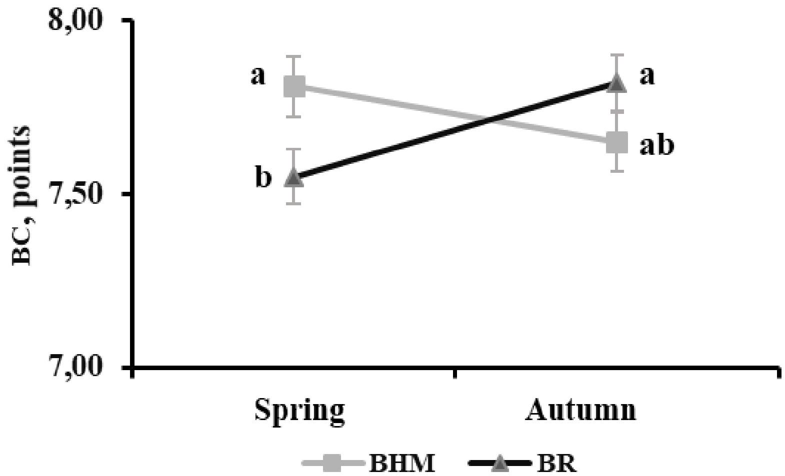
WPB= body weight; MW= metabolic weight; APB= age; BS= body surface; WPB/BS= body weight between body surface; MW/BS= metabolic weight between body surface; BC= body condition; DM= depth of the long dorsal muscle; DF= thickness of dorsal fat; LEP= serum leptin concentration. *Results of the second year.

[a,b] Means with different literal between columns of each fixed effect in each of the response variables indicate difference (*P*<0.05).

Effect of breed x season of birth interaction on body condition

BR heifers born in spring had lower BC than BR heifers born in autumn and BHMs born in spring (*P*<0.05) but were similar to BHMs born in autumn (Figure 1).

Figure 1: Least squares means and standard errors for body condition (BC) at puberty, by breed and season of birth



BHM= Brahman, BR= Braunvieh. Different literals (a,b) between means indicate difference (*P*<0.05).

It was observed that 48 of the 65 BHM heifers started PB in the months in which light hours increased (December 22 to June 21) and the other 17 heifers did so in the months in which light hours decreased (June 22 to December 21) (*P*<0.05). On the contrary, BR heifers began PB regardless of the trend of change of the photoperiod, as 30 of them began PB when light hours increased and another 26 did so when light hours decreased (*P*>0.05).

Discussion

The present study provides relevant information on the relationships between markers of body composition, the internal environment of animals and those of the external environment with the establishment of puberty in heifers. The relationship of the establishment of PB with body characteristics such as the amount of fat and BS is highlighted; for the latter, the difference shown between breeds (17.8 %) reduced when incorporating WPB/BS (6.4 %) in the analysis, but disappeared when considering MW/BS (<1 %; *P*>0.05), which suggests that more than BS itself, the important indicator is the relationship of body mass with BS, regardless of breed, despite the clear and significant ($P<0.05$) differences at PB in the rest of the variables studied between BHM and BR. To a large extent, the differences in several body characteristics between BHM and BR at PB could be associated with the fact that PB in BHM occurred with 4.6 mo more in age, a period in which they continued to grow and modify their body composition, however, there was no difference in the MW/BS ratio at PB, which suggests that this is an important parameter; if it is not a trigger, it is at least indicative that there is an energy balance that allows covering the demand for vital functions, thermoregulation, locomotion, growth, and there is a surplus to be allocated for reproductive processes[19].

The treatment with zilpaterol to half of the animals to induce a different body composition between them and those of the control group allowed observing that, despite the differences at PB in terms of the variables weight, age and body characteristics, due to the treatment, DF and LEP did not show differences between the ZIL and control groups, which highlights the importance of body composition and, in particular, the amount of body fat for the establishment of PB.

It has been reported that in the growth and composition of the carcass, there is a large variation between *B. taurus taurus*[20,21] and *B. taurus indicus*[22]. Likewise, there are differences in the distribution of body fat between breeds; dairy breeds deposit a higher proportion of their fat internally and a smaller proportion subcutaneously than beef breeds[23]. These differences, regardless of environmental factors, apparently participate in the maturation processes so that individuals are chronologically different between breeds[24], with the consequent variation in age at first calving, which is a variable of economic importance. The estimated age at the PB of Zebu cattle in the tropics and subtropics ranges widely between 16 and 40 mo[25] and, consequently, the age at first calving is also very variable due to the effect of environmental variables, with the access of feed to the animals standing out, which in grazing conditions depends on conditions such as rainfall, the quality and fertility of soils and forage species in use, particularly in tropical conditions, so that the study of physiological phenomena, such as the establishment of puberty and seasonal effects on reproductive processes, require the control of variables such as quantity and quality of feeding, which can confuse the results. For that reason, for this work the animals were individually fed so that they all had a similar daily gain. The overall average daily weight gain was 560 ± 121 g/d.

At PB, BHM heifers were heavier and older than BR; in fact, those 142 d of difference implied that BHM had on average 74.8 kg more (in congruence with the average weight gain of the heifers during the study), however, due to the difference in BS, there was no difference in MW/BS between the two breeds. A correlation of 0.96 ($P<0.01$) between WPB and BS was observed. Other researchers[26-28] have reported that *B. taurus indicus* heifers require greater weight and age to begin PB than *B. taurus taurus* heifers. In addition, it has been described[29] that, in Nelore heifers, a good post-weaning feeding is an effective method to accelerate PB. Among *B. taurus indicus* breeds, Nelore, which is the one that apparently has fewer flaps and folds of skin and, therefore, less body surface, has had greater popularity in Brazil, apparently, due to its better productive performance, particularly, greater precocity to begin PB[29] and lower age at first calving[30,31]; however, no studies that specifically linked these reproductive characteristics to the BS of animals were found.

The importance of the relationship between body surface and mass in the energy cost of maintenance of animals has been recognized and considered for more than a century; in fact, among the initial studies on basal metabolism, it was discussed that BS was as important or more important than body mass[32]. In studies conducted with rodents, canines, cattle and humans, it was observed that BS is a variable that allows the most accurate prediction of metabolic rate[33]. Therefore, it was considered as the variable that allowed, with greater precision, the comparison between animal species of different sizes in quantitative metabolism studies[34], which promoted the development of instruments for its measurement[35]. No studies specifically designed to associate the BS of cattle with some productive characteristics were found; in previous studies[36,37], the BS of dairy cows and its relationship with body weight were measured, for the use of a formula based on "Kleiber's Law" in bioenergetics studies.

It has been estimated that at a higher BS, the loss of body heat by radiation, convection and evaporation[38] increases, conditions that imply an energy cost. It has been evaluated that from birth, as the body mass of the animal increases with growth, its relative proportion to body surface also increases, and the relative dissipation of body thermal energy reduces, gradually allowing more energy available for physiological processes and the storage of surpluses. Among those physiological processes not fundamental to sustain the life of the individual is reproduction, which becomes feasible once the energy available in the organism guarantees the processes indispensable for life and other priorities such as locomotion. This requires a balance between the energy that is consumed and processed and that which accumulates in tissues, to then be transformed into work or dissipated into the environment as caloric energy; in the latter function, skin and respiration play a central role in cattle[19].

In the case of *B. Taurus indicus* cattle, their greater adaptation to hot climates, such as tropical ones, is due to their superior ability to regulate body temperature during heat stress conditions, derived from a lower metabolic rate and greater ability to dissipate heat through the skin[39]. It should be remembered that animals that have a higher proportion of Zebu genes show smaller size of their thoracic and abdominal organs, such as rumen weight and length of intestines than *B. taurus taurus* animals[40,41]. It has been observed that the basal metabolic rate in *B. taurus indicus* x *B. taurus taurus* is less than in *B. taurus taurus*. In one study, the rate of heat produced per unit body surface of non-lactating and fasting cows was 57 MCal/m^2 for Red Sindhi x Holstein and 100 MCal/m^2 for Holstein[(42)].

From a practical point of view, a larger skin surface in Zebu, on the one hand, confers advantages (heat dissipation and resistance to thermal stress), but, apparently, it is associated with lower efficiency in the use of food energy[(38)], growth rate[(39)] and accumulation of energy in the form of body fat[(43,44)], which is an important factor for PB, as reiterated in the results of this work, where, despite the use of zilpaterol, which promoted faster growth, puberty was reached at an older age, with greater weight and musculature in the treated heifers, but with similar DF between controls and treated.

Supplementation with β-agonist ZIL caused APB to increase, and the control heifers to be younger at PB. The administration of ZIL modifies the distribution of nutrients, so that animals grow faster and gain more weight, but this gain is leaner[(45,46)]. For the beginning of PB, not only is absolute weight gain of heifers important but also body mass composition[(47-49)], as subtle or acute changes in metabolic state are likely to begin physiological events leading to puberty[(5,50)]. Hence, although those treated with ZIL reached with more weight and APB, they were not different from those of the control group in DF, despite the fact that the latter were lighter and with less DM, which was one of the assumptions of the study on the effect of β-agonists in the distribution of nutrients, causing decreased lipogenesis and increased muscle accretion, as observed by several authors, who have reported that ZIL supplementation increased DM and decreased DF in cows[(51)], heifers[(52)] and steers[(53)].

The available information indicates that a minimum of body fat is required to trigger reproductive processes such as PB in heifers[(9)] and a minimum of BC[(54)]. In a study[(55)] conducted on Nelore heifers, high correlations (from 0.82 to 0.93) between BC and DF were found, and it was stated that with BC, DF can be predicted in *B. taurus indicus* cattle at different stages of the production cycle. In a study with *B. taurus taurus* cows[(56)], it was observed that an increase in BC tended to be accompanied by an increase in the size of adipocytes in subcutaneous adipose tissue. It was observed that *B. taurus indicus* animals needed more fat accumulation than *B. taurus taurus* to begin PB, which can be induced by feeding higher energy diets. In this study, in both breeds these relationships were modified by the use of β-agonist ZIL; since the heifers treated were more muscular and had higher BC and more DM at PB, as expected[(5)], but there was no difference with those of the control group in DF at PB.

It is evident that in BHM, above the internal markers related to weight and body composition, a seasonality effect associated with light changes prevailed; therefore, an effect of BD on APB and differences between breeds in the months of occurrence of PB were observed. The seasonality effect was manifested in that BR heifers started PB homogeneously throughout the year, but 73.8 % of BHM heifers started PB in the months when light hours were increasing and only 26.2 % did so when light hours decreased ($P<0.05$). In fact, the effect of seasonality is manifested on other variables, as shown in Table 2, where the differences in variables that was found as critical for the beginning of PB, such as MW/BS, DF and LEP, remained in animals born in different seasons. The observation of the effect of seasonality on PB in BHM coincides with that described by other authors(3), who observed that BHM heifers only started puberty in a period from February to May (days in which light hours increase), unlike Brown Swiss heifers that began puberty throughout the year. This effect was attributed to the susceptibility of BHM heifers to the environmental effects determined by the seasons of the year (seasonality). These observations coincide with results of other studies with Zebu females(11,12,57), where a seasonal trend in the postpartum reproductive activity of cows was observed, even under controlled feeding conditions. The photoperiod seems to influence the beginning of puberty. In an experiment with dairy heifers, supplemental lighting (16 h of light/day) during the winter improved growth rates and reduced age to puberty(58). Similarly, with *B. taurus taurus* heifers, supplemental lighting (18 h of light/day), after 22 or 24 wk of age, reduced the age to puberty in heifers born from February to July. These photoperiod effects were accompanied by changes in ovarian development(59). The same author(60) points out that heifers with a genetic propensity to reach puberty at an early age may be affected by the season of birth differently than those who reach puberty at older ages.

Compared to BR heifers, BHM heifers were more susceptible to environmental signals from the change of light hours, which probably triggered the neuroendocrine processes associated with the beginning of PB, a situation that should be considered in the planning of reproductive management programs for cattle in herds with a diverse breed composition. The effect of the season of birth influenced that heifers born in autumn had higher DF and higher levels of LEP at PB than those born in spring, but there were no differences ($P>0.05$) in APB between seasons of birth under the conditions of this study.

Interaction of BD x SB on BC was observed at puberty, where BR heifers born in spring showed lower BC than BR heifers born in autumn and BHMs born in spring. Based on general averages (not shown here, they can be consulted in a previous study(61)), apparently, the heifers of lower APB (BHMs born in spring and BRs born in autumn) reached with greater BC (Figure 1), although the differences in this variable were minimal. In this regard, several authors have suggested that there is a phenomenon of compensation of age with weight for the establishment of PB(49,62,63).

It has been reported that in *Bos taurus taurus* cows(56), there is a positive correlation of leptin with body weight and BC; in addition, an increase in BC tended to be accompanied by an increase in the size of adipocytes in subcutaneous adipose tissue. In the present study, LEP at PB was different between breeds; BHM heifers showed higher LEP than BRs. This difference in LEP between breeds may be due to the fact that the BHMs were the ones that also presented the greatest age and amount of DF at PB, since the circulating levels of leptin are directly associated with body adiposity[64,65]. So far there is no evidence of an abrupt transition in prepubertal plasma concentrations of leptin at puberty or that circulating concentrations may be a critical trigger for PB in fast-growing heifers, but apparently a minimum of circulating leptin is required for PB in heifers with normal or restricted growth rates[66].

Conclusions and implications

It is concluded that body mass as a proportion of BS appears to have an important role in the beginning of PB in heifers and that a minimum mass of metabolically active tissues per unit of BS and a minimum of fat accumulation are required for the beginning of PB to be triggered, regardless of the breed in question. The establishment of puberty is a complex phenomenon, which depends on the interaction between variables and internal markers of animals and other factors in their environment, such as changes in light hours, which affect Zebu heifers. The observations derived from this work allow speculating that the late puberty of BHM heifers may be associated with a higher BS compared to BR heifers and confirms the effect of changes in light hours on reproductive phenomena in Zebu females, even when the indirect effect of seasonality is eliminated through controlled feeding. This work documents for the first time the relationship of body mass, as a proportion of body surface, with the establishment of puberty in cattle.

Conflict of interest

The authors declare that there is no conflict of interest in the presentation of this work.

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