16 HOURS PHOTOPERIOD IN HOLSTEIN HEIFERS IN THE SUBTROPICS: EFFECTS IN DEVELOPMENT AND AGE TO FIRST ESTRUS

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ABSTRACT. Both growth and milk production in heifer calves are stimulated by a long photoperiod, though this has not been proven yet in subtropical areas. To evaluate the effect of 16 hr of light (L16) on calves suckling (LAC) and/or pre-pubertal stages (PP) in a subtropical area, 325 calves (36 ± 0.4 kg) were randomized into two groups: L16 or natural photoperiod (LNA T). At seven months of age, 198 of these calves (195 ± 2 kg) were randomly assigned to L16 or LNA T. The following were determined at the beginning and end of LAC and PP stages: Weight (PC, kg), height (AC, cm), thickness of back-fat (GG, cm) and of Longissimus dorsi (LD, cm); pelvic area (AP, cm²), body condition (CC), age at first estrus, mammary gland depth (PGM, cm) and width (AGM, cm). The statistical analysis was made using ANOVA for a 2 X 2 factorial arrangement. At the end of LAC, GG was lower and LD greater in L16 animals, while, at the end of PP, L16 heifer calves had a greater PC (260 ± 3 vs 250 ± 3), AP (166 ± 1.2 vs 153 ± 1.2), LD (3.18 ± 0.04 vs 2.90 ± 0.04), and AGM (2.41 ± 0.02 vs 2.21 ± 0.02), but a lower GG (0.114 ± 0.003 vs 0.139 ± 0.003) as compared to LNA T heifer calves. More L16 animals (67 %) presented estrus versus LNA T (38 %) and the age of the first estrus was lower in L16 (278 ± 2 vs 288 ± 2 d). Consequently, exposure to L16 during LAC promotes lean growth while lean growth and a bigger pelvis and mammary gland are prompted during PP. Hence, it may be concluded that PP heifer calves exposed to L16 have a higher production potential and lower risk of dystocia than LNA T animals.

Key words: Photoperiod, development, heifers calves, estrus, subtropics.
INTRODUCTION

The generation of replacement heifers is a key operation to achieve maximum efficiency in milk production systems because it represents the second largest economic cost, only below the cost of feeding dairy cows (Heinrichs 1993, Vandehaar 2001). The rate of weight gain is very important to ensure the weight, age and body composition suitable for reproduction and for optimal mammary development (Sejrsen et al. 1982, Zanton and Heinrichs 2005). The development of the mammary gland can be affected by environmental factors, the most prominent being nutrition and photoperiod, which determine the expression of the genetic potential of the heifers (Sejrsen et al. 2000, Dahl and Petitclerc 2003, Collier et al. 2006).

Photoperiod manipulation can be a useful tool to increase production efficiency in cattle; in some studies conducted in countries geographically situated between 37° and 62° N (Dahl et al. 2000, Dahl and Petitclerc 2003, Auchtung et al. 2005), it has been proved that a light regime of 16 h of light and eight of dark, applied during lactation to Holstein cows, increases milk production by 12 to 15 %, compared with a system of eight hours of light and 16 h of darkness. Regarding prepubertal heifers, an 16 h of light regimen held for 270 d, advanced the onset of puberty and increased the height and the mammary gland development in comparison with animals kept in 16 h of darkness (Rius et al. 2005, Rius and Dahl 2006). Likewise, heifers exposed to a long photoperiod had an increased growth rate and height, and exhibited a lean format of growth and a higher milk production in their first lactation compared to animals exposed to a short photoperiod (Tucker et al. 1984, Rius et al. 2005, Rius and Dahl 2006). Endocrine mechanisms, regulating growth and milk production as a response to long photoperiod in bovines, are becoming clearer, however the information is still fragmentary. In cattle, as in most of the mammals studied, the response to photoperiod is dependent on melatonin secretion from the pineal gland (Lincoln et al. 2003), also called photoperiod hormone (Goldman 2001), which indirectly influences secretion of growth hormone (GH) (Kendall et al. 2003, Jin et al. 2012) and prolactin besides modulating endocrine effects that affect growth, reproduction and lactation (Lincoln et al. 2003). After melatonin, the more consistent endocrine response caused by the photoperiod is the prolactin secretion (Dahl et al. 2000, Lincoln et al. 2003), increasing during the long days and decreasing during the short days (Peters et al. 1980, Dahl et al. 2000, Freeman et al. 2000). Cows and heifers exposed to 16 h of light also increase their serum concentration of the growth factor similar to insulin type 1 (IGF-1) (Dahl et al. 1997, Akers et al. 2005, Spicer et al. 2007), which is an essential component of multiple systems of body growth and metabolism regulation during pre and postnatal periods (Le Roith et al. 2001) as well as the mammary gland development (Akers et al. 2005). The increase of circulating IGF-1 caused by the long photoperiod is independent of GH (Dahl et al. 2000) and of the expression of GH receptors in the bovines’ liver cells (Kendall et al. 2003) Evidence show that increments in the circulating IGF-1 an indirect action of prolactin which inhibits expression of the binding protein to IGF -1 number 5 (IGFBP-5) in liver (Rosato et al. 2002) and mammary gland (Accorsi et al. 2002, Dahl and Petitclerc 2003), which prevents the IGF-1 to exert its positive effects on cell proliferation, gene expression of casein and glucose transporters and apoptosis inhibition (Flint et al. 2005). The effects of the long photoperiod, raise of prolactin and IGF-1 circulating levels and the decrease of IGFBP-5, induce a higher bone and skeletal muscle growth (Salih et al. 2004) along with mammary development with a high proportion of parenchyma than stroma (Petitclerc et al. 1985, Tonner et al. 1997).

Previous studies of the effects of long photoperiod in dairy cows and heifers have been made in latitudes between 37° and 62° N (Dahl et al. 2000, Auchtung et al. 2005), where differences in the natural photoperiod duration are remarkable between summer and winter months. In the Laguna region of Coahuila, located at 26° 23’N (subtropical region), it was demonstrated that the 16 h light regime increased by 25 % the milk production in adult goats (Flores et al. 2011); consequently, it
is also possible that the difference between 16 h light and natural photoperiod in this region is perceived by the brain of bovines, which reacts by adjusting physiological mechanisms that promote accelerated and lean body growth, as well as a higher mammary gland development. With this, the animals would have increased production in their first lactation. In this process the attention is focused in two critical periods of development: the preweaning and pubertal stages. It has been proven that during the preweaning period the development can be induced preferentially towards further growth of the parenchyma in relation to adipose tissue by increasing the levels of protein and energy in the diet (Brown et al. 2005); in contrast, in prepubertal stages, when there is an accelerated growth of milk ducts and the terminal duct units, structures that are the precursors of the lobule-alveolar system (Capuco and Ellis 2013), the energy and protein rise in diet, promotes mammary growth towards a higher adiposity (Brown et al. 2005). However, a photoperiod of 16 h exposure induced development of the mammary parenchyma (Petitclerc et al. 1985) in prepubertal calves and heifers that were cycling.

To date, in the available scientific information, there have not been found any cows or heifers works done in tropical or subtropical regions where the effects of long photoperiod on mammary growth and development are assessed. Therefore, the aim of this study was to determine the effects of a light regime of 16 h light, held for 60 consecutive days, during preweaning and/or prepubertal periods, on body and mammary development and the age at first estrus of replacement Holstein calves, in a region of the Mexican subtropics.

**MATERIALS AND METHODS**

The procedures performed in this study were approved by the Institutional Subcommittee on Care and Use of Animals in Experimentation (Graduate Program, Faculty of Veterinary Medicine, UNAM).

The study was conducted on a dairy farm in the municipality of Gómez Palacio, Durango, located at 25° 41' 42'' N and 103° 27' 43'' O, and was carried out in two stages:

**Preweaning stage (PW).** 325 newborn Holstein calves were sheltered in a completely covered maternity pen with wind protective curtains. The facility has 450 individual cages equipped with bucket racks for two pails in which milk and water are provided. In addition, the cages contain an automatic feeder for forage and concentrate. The cages have slatted floor with comfortable rubber mats, with holes that allow the passing of calves ejections to lower pits, which are washed by flushing water. Calves were assigned according to a random block design to two treatments (block criteria: group of calves entering the maternity area within a period of 40 d): a) Extended photoperiod of 16 h light (L16, n = 163), and b) Natural photoperiod (LNAT, n = 162) (Figures 1 and 2). The place was divided using tarpaulins that prevented the passage of light from the lamps used in the subdivision of the calves of L16 to the subdivision that housed animals in LNAT; LNAT animals were kept with natural light for 60 d (birth to weaning), while those of L16 received additional light for the same period. In the area of the maternity pen that housed calves in L16, "Metal-Halide" lamps (mixture of vaporized gases of mercury with bromine or iodine) were installed, illuminating the entire area with at least 450 lux for 16 h d⁻¹, measured to the eye level of the animals regardless of their position (Rius and Dahl 2006). The evening lighting of the lamps were scheduled daily to complete 16 h of light. The daily duration of h light (Figure 1) for the latitude of the experimental site was taken from an online software for photoperiod calculation (Lammi 2011) and the lamps were programmed to light 0.5 h before the time set as the twilight, in order to ensure that the natural light would not decline below 150 lux, lowest intensity limit of detectable light for cattle (Dahl, 2005). In both sections, at the beginning and every third day of the experiment, measurements of the lux units were made by the use of a light meter (LT-1108 Lutron Electronic Enterprise Co. Taiwan). Measurements were made immediately before and after the lighting of lamps and at 22:00 h. For routine monitoring of calves at night, a hand lantern with infrared light (less than 5 lux) was used because it has no effects in animals (Drouyer et al. 2007).
Calves feeding was performed according to the barn management program; immediately after birth and 12 h later, the calves consumed at least two litres of pasteurized colostrum (post-pasteurization concentration of immunoglobulins > 50 g L\(^{-1}\)). From the second day after birth and until weaning, calves of both treatments were fed with four liters of pasteurized milk per day. From day five of life, animals received ad libitum water and calf starter (27.67 % of crude protein, 2.95 % of crude fat and 2.83 Mcal kg\(^{-1}\) of metabolizable energy; Nuplen\(^{\text{R}}\) SA de CV, Santiago Papasquiaro, Gómez Palacio, Durango, Mexico).

Prepubertal stage (PP). Approximately at seven months of age (230-250 d), 198 of the animals used in PW were randomized into two barnyards, one with L16 and the other with LNAT, according to a 2 x 2 factorial arrangement (two physiological stages: PW and PP; two photoperiod: L16 and LNAT), leaving in each of the two PP groups the 50 % of animals that during PW were exposed to L16 and the rest 50 % that during PW remained on LNAT. Consequently, the treatment combinations were LA CL16-PPL16, LA CLNA T-PPL16, LA CL16-PPLNA T and LA CLNA T-PPLNA T. Both PP groups were divided into two treatments for 60 d: a) Artificial Photoperiod of 16 h light (L16; n = 99), and b) Natural Photoperiod (LNAT; n = 99).

The barnyards had enough space to reduce the social competition between heifers and they were 30 m apart from each other. The barnyard where LN heifers were housed was all the time under LN and the barnyard for heifers in L16 was entirely lit using "Metal Halide" lamps, providing at least 450 lux from the roof to a height of 20 cm above the soil surface. As in PW, lamps were programmed to turn on for the required amount of hours to complete 16 h of light per day according to the data obtained from the natural photoperiod of Lammi (2011) (Figure 2). Twice a day it was offered the same integral ration to both groups, which consisted of sorghum silage, corn stover, alfalfa hay, canola paste and mineral salts mix. Heifers remained 60 d under the described treatments.

In both stages, PW and PP, it was recorded the height at the withers (WH) and body weight (BW) at the start and end of the photoperiod treatment (Rius et al. 2005). With the same frequency the thickness of the backfat (BFT) and the depth of the Longissimus dorsi muscle (LD) was recorded by ultrasound (Titan-SonoSite \(^{\text{C}}\), SonoSite Inc. USA; sectorial probe of 5-10 MHz) at the intersection of the thoracic and lumbar regions (Bailey et al. 1986). To determine the effects of light on mammary development, the right rear quarter of the udder was examined by ultrasonography (Franz et al. 2004), where depth (MGD) and width (MGW) of the gland were recorded. Additionally, the pelvic growth in heifers was evaluated by using a Rice pelvimeter (Rice Pelvimeter, Lane Manufacturing Inc. USA) to calculate the pelvic area (PA) (Van Donkersgoed et al. 1990), and body condition was measured (BC, scale of one to five), using the proposed scale by Edmonson et al. (1989).

Age at first estrus was determined as an indicator of the beginning of puberty, by observing and recording behavioural signs of it (0600-0800 and 1800-2000 h). Estrus detection was carried out daily by a single person from the beginning to the end of the light treatments and as a heat detection aid crayon labeling on the tail head of heifers was used. Heifers which stood for mounting by a partner for at least three seconds, were considered in estrus; all of the heifers identified in estrus by the method of crayon were detected in estrus by the observer. Furthermore, the daily feed intake (DFI) was estimated by barnyard and individual daily weight gain (DWG) was calculated.

Data analysis
Statistical analysis of the response variables recorded in PW was performed using an analysis of variance for randomized block designs. For this, the GLM procedure of SAS statistical software (SAS 2009) was used. Data from feed intake in PW calves were analysed by analysis of variance for split plot designs with repeated measures in time; for this it was applied the MIXED procedure of SAS statistical software.
**Figure 1.** Natural photoperiod (solid line) at the farm where the study was performed (25° 41’ N, 103° 27’ W), during intervals of calves exposure to 16 h light (segmented line) or natural photoperiod. In insets dates of entrance (birth) and exit (weaning) of animals to exposure to light regimes are shown. Animals were grouped in two random blocks (rectangles).

**Figure 1.** Fotoperíodo natural (línea continua) en el establo donde se efectuó el experimento (25° 41’ N, 103° 27’ O), durante los períodos de exposición de becerros a un régimen de 16 h (línea segmentada) luz o fotoperíodo natural. En los rectángulos se indican las fechas de inicio (nacimiento) y fin (destete) del período de exposición de los animales a los regímenes de luz. Las becerros ingresaron al estudio en dos bloques completos al azar.

**Figure 2.** Natural photoperiod (solid line) at the farm where the study was performed (25° 41’ N, 103° 27’ W), during the exposure of prepuberal heifers to a 16 h light regime (segmented line) or natural photoperiod. In insets dates of entrance (233 ± 20 d old) and exit (293 ± 20 d old) of animals to the experiment are shown. Animals were grouped in randomized blocks (rectangles).

**Figure 2.** Fotoperíodo natural (línea continua) en el establo donde se efectuó el experimento (25° 41’ N, 103° 27’ O), durante los períodos de exposición de vaquillas prepuberiles a un régimen de 16 h luz (línea segmentada) o fotoperíodo natural. En los rectángulos se muestra la fecha de ingreso (233 ± 20 d de edad) y salida (293 ± 20 d de edad) de los animales a el experimento en forma de bloques completos al azar.
RESULTS

LAC stage

At the moment of birth there was no difference \((p > 0.05)\) between groups. At weaning BFT was lower \((p < 0.001)\), but LD and the conversion efficiency were higher \((p > 0.001)\) in calves LAC-L16 (Table 1). The average daily intake of calf starter of calves in LAC-LNAT was higher \((p = 0.001)\).

PP stage

At the beginning of the second exposure to the regimen of light \((233 ± 20\text{d of age})\), there were differences \((p < 0.05)\) for BC, PA and BFT, that is why those values were used as covariates to analyse the corresponding data recorded at the end of the PP period \((293 ± 20\text{d of age})\). At the PP end, L16 heifers had more \((p < 0.05)\) BW, LD, PA and MGW, but less \((p < 0.05)\) BFT than LNAT heifers (Table 2).

Feed intake per barnyard during PP was similar \((p > 0.05)\) in both groups, being the average intake of all the heifers of 15.44 ± 0.13 kg d\(^{-1}\) (wet base). Meanwhile, it was observed that the age of the first heat occurred earlier \((p < 0.05)\) in heifers in L16 \((278 ± 2 \text{d})\) than in heifers of LNAT \((288 ± 2 \text{d})\); furthermore, within the L16 group a bigger percentage \((p < 0.05)\) of the heifers (67 %; 67/99) had their first estrus during the 60 d of the light treatment, in comparison with the animals in LNAT (38 %; 38/99). Finally, when performing the analysis according to the factorial arrangement, no significant interactions were found \((p > 0.05)\) between growing stages (LAC and PP) and photoperiod treatments (L16 and LNAT) in any of the response variables studied.

DISCUSSION

During PW, the final weight was similar between groups, observation that differs from what was found by Osborne et al. (2007), who documented that Holstein calves from 1 to 8 weeks of age exposed to 18 h light, were heavier than calves maintained under 10 h light. The contrasting results between the present study and that of Osborne et al. (2007) could have been due to different ambient temperatures that prevailed in the studies, since ours began in fall whereas Osborne et al. (2007) initiated in summer, and it was documented that prolactin release increases during spring and summer but declines during fall and winter (Peters and Tucker 1978), regardless of a similar but mechanistically independent effect induced by seasonal variations in temperature (Wetteman et al. 1982); consequently, the previously mentioned effects of prolactin on IGFBP-5 and IGF-1 could be negligible in calves in the present study relative to those observed in Osborne et al. (2007). That is possible because in this experiment the recorded temperatures were as low as 6 °C, whereas in Osborne et al. (2007) animals were maintained under constant temperature of 20 °C; relative to this effects, Yaegashi et al. (2012) reported that prolactin was secreted at a lower rate in goats exposed to L16 and at 5 °C than goats at similar light regime but at 20 °C. In contrast with the difference in body weight, height of animals in this and Osborne et al. (2007) studies was not affected by photoperiod during PW, thus the only two published works conducted in preweaned calves, this and Osborne et al. (2007), indicate that L16 increases does not affect height in the subtropics or any other latitude during the first two months of the calves life; however, both studies with preweaned calves indicate that exposure to L16 increases the efficiency in nutrients utilization, since in the present experiment calves under L16 recorded higher body weight gain by unit of calf starter intake than in Osborne et al. (2007). Exposure to L16 induced a greater ADG than in calves maintained in a short photoperiod. The highest feed efficiency evoked by the long photoperiod in preweaned animals has been associated with a faster rumen development, as it was evidenced by the early increased concentration of blood volatile fatty acids and higher ACG in calves exposed to L16 (Osborne et al. 2007). Similarly, seasonal variations were observed in gastrointestinal structures and their functions, as well as in patterns of nutrients absorption (Rhind et al. 2002); changes that may contribute to variations in metabolism, hormonal signaling ac-
tions along with the interpretation of these signal at the neuronal centers that regulate voluntary intake. Relative to this, evidence exist that the hypothalamus receives information associated with the organism nutritional and metabolic conditions via signals from peripheral tissues such as insulin, leptin, ghrelin (Sartín et al. 2011), adiponectin (Alonso-Vale et al. 2009) and several intestinal peptides (Konturek et al. 2011). Associated with the activation or inhibition of the orexigenic and anorexigenic pathways at brain level (Valassi et al. 2008), which could explain at least partially, the lower feed intake during PW period in calves exposed to 16L relative to those of under LNAT. On the other hand, GH, IGF-1 and prolactin have been implicated in promoting gastrointestinal growth in non-ruminant animals (Rhind et al. 2002); indeed, it has been demonstrated the expression of receptors for IGF-1 in the intestine of mice (Dong et al. 2011), as well as for GH and prolactin in all segments of the intestinal epithelium of humans, rabbits and rats (Nagano et al. 1995), findings that permit to suggest regulatory actions of these hormones as a result of photoperiodic variations at gastrointestinal level. Moreover, the documented profound effects of photoperiod on development and functions of the gastrointestinal tract (Rhind et al. 2002, Konturek et al. 2011), makes feasible to propose effects of melatonin as an activator of peripheral oscillators at stomach, liver and other digestive organs level, as well as an inhibitor of oxidative and nitrosative stress, actions that affect intestinal motility, gastric secretions, proliferation of gastroenteric epithelium, production of digestive enzymes, nutrient transport across small intestine epithelium, as well as modulation of the immune system inherent to the gastrointestinal tract (Konturek et al. 2011). The results of this study along with others (Peters et al. 1980,

### Table 1. Body measurements of heifer calves at the beginning (birth) and end (weaning) of exposure to 16 h light (L16) or natural photoperiod (LNAT).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Birth</th>
<th>Weaning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L16</td>
<td>LNAT</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>36.800 ± 0.40</td>
<td>36.800 ± 0.40</td>
</tr>
<tr>
<td>Height to the withers, cm</td>
<td>75.500 ± 0.30</td>
<td>75.100 ± 0.30</td>
</tr>
<tr>
<td>Dorsal fat, cm</td>
<td>0.076 ± 0.02</td>
<td>0.078 ± 0.08</td>
</tr>
<tr>
<td>Muscle depth, cm</td>
<td>1.820 ± 0.30</td>
<td>1.810 ± 0.30</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

*a,b* Different literal in the line, between treatments and in the period, indicates statistic difference (p < 0.001).

### Table 2. Body measurements in prepuberal heifers at the beginning (233 ± 20 d of age) and end (293 ± 20 d of age) of exposure to 16 h light (L16) or natural photoperiod (LN).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Beginning</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L16</td>
<td>LN</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>195.000 ± 2.000</td>
<td>260.000 ± 3.000 a</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.080 ± 0.003</td>
<td>1.170 ± 0.002</td>
</tr>
<tr>
<td>Pelvic area, cm²</td>
<td>141.100 ± 1.100 a</td>
<td>133.200 ± 1.100 b</td>
</tr>
<tr>
<td>Body condition, point</td>
<td>2.700 ± 0.020 a</td>
<td>2.600 ± 0.020 b</td>
</tr>
<tr>
<td>Dorsal fat, cm</td>
<td>0.113 ± 0.002 a</td>
<td>0.122 ± 0.002 b</td>
</tr>
<tr>
<td>Muscle depth, cm</td>
<td>2.880 ± 0.020 b</td>
<td>2.880 ± 0.020 a</td>
</tr>
<tr>
<td>Mammary gland depth, cm</td>
<td>0.540 ± 0.006</td>
<td>0.540 ± 0.006</td>
</tr>
<tr>
<td>Mammary gland width, cm</td>
<td>2.000 ± 0.030</td>
<td>2.000 ± 0.030</td>
</tr>
</tbody>
</table>

*a,b* Different literal between columns, within the period, indicates statistic difference (p < 0.05).
Petitclerc et al. (1983), where weaned, prepubertal heifers exposed to L16 had a higher feed efficiency than heifers under shorter photoperiod regimes, provide bases to propose that an extended photoperiod is an effective management tool to modify the metabolism in PW calves and PP weaned heifers in such a way that growing becomes a more efficient process under conditions that prevail in subtropical regions or in localities situated north of the Tropic of Cancer, by altering the physiological mechanisms discussed in the previous paragraph. It is convenient to emphasize that L16 promoted lean growth in PW and PP animals despite variations on natural photoperiod (Figures 1 and 2), thus within the photoperiod range in the locality, additional light seems to be a sufficient stimulus to trigger mechanisms that direct toward a lean growth format from fall through summer.

Regarding the PP stage, several workers reported that exposure to a long photoperiod, relative to natural photoperiod or exposure to 8 h light, promotes growth in Holstein heifers (Peters and Tucker 1978, Petitclerc et al. 1983, Tucker et al. 1984, Zinn et al. 1986, Rius and Dahl 2006), consequently, the information in this work relative to the BW increment in heifers is in agreement with data previously published over the effects of L16 in locations above the Tropic of Cancer; our findings allow to say that the effects of a long photoperiod are exerted similarly in subtropical areas than in latitudes above 37° N.

In this study it was observed that relative to natural photoperiod, L16 modified body growth composition, since that light regime reduced the back fat depth and increased density of the Longissimus dorsi muscle during PW and PP stages. To our knowledge, there is no precedent on the influence of L16 in preweaned calves body composition; however, in PP weaned heifers some conflicting results have been reported, for instance while Petitclerc et al. (1984) found that L16 promotes protein accretion in skeletal muscle and 8 h light increased fat deposition, Zinn et al. (1986) observed a similar L16 effect in muscle exclusively in pubertal but not in prepubertal heifers. Therefore, data from the present study give support to the findings of Petitclerc et al. (1984) and provide evidence to propose that exposure of prepubertal heifers to L16 promotes lean body growth from birth to at least nine months of age. On the other hand and according to our knowledge, this is the first time that the positive effect of L16 on pelvic area, an important characteristic associated with the maternal calving ease trait. Potentially the influence of exposure to an extended light regime may reduce the probabilities of dystocia at first calving. What are the physiological mechanisms that mediate effects of L16 on pelvic area? It has been determined that lengthening the daylight increases the circulating levels of IGF-1 in Holstein cows and heifers (Akers et al. 2005, Dahl et al. 1997). Likewise it was established that the concentration of plasma prolactin rises as length of the daytime period is increased (Peters et al. 1980, Dahl et al. 2012). The interaction of these two hormones along with the reduction of IGFBP-5 in blood and several body tissues is the main mechanism proposed in this study, as responsible for the changes in body composition and the bone increment (pelvic area) observed in animals. It is known that IGF-1 is a protein that promotes growth and remodeling of bone (Canalis 1993), and participates in modulating cell survival as well as development of tissues such as muscle and mammary gland parenchyma (Coolican et al.1997, Akers et al. 2005). Other researchers found that IGF-1 stimulates bone formation, by regulating proliferation, differentiation and survival of osteoblasts (Grey et al. 2003). In support of this, bone formation is severely compromised in mice with a disrupted IGF-1 gene (Liu et al. 1993). A key component in the regulatory role of IGF-1 actions in bone and muscle is IGFBP5. For example, transgenic mice overexpressing IGFBP5 compared with wild type mice, had a reduced litter size and the surviving offspring had a lower birth weight and a reduced growing rate; likewise pups of overexpressing IGFBP5 mice had a retarded skeletal muscle development (Salih et al. 2004) as well as deficiencies in bone volume and density (Devlin et al. 2002). In turn, prolactin inhibits synthesis and release of IGFBP5 in several tissues (Tonner et al. 1997), promoting with this action a greater availability of circulating and lo-
cal IGF-1, and a reduction of the negative effects exerted by IGFBP5 in tissues which are described below.

As for mammary gland development, in the present work L16 heifers had a greater width than animals under natural photoperiod, indicating that the long photoperiod induces mammary growth. It can be speculated that long photoperiod induces mammary growth by privileging growth of parenchyma over adipose tissue, as it promotes lean body growth. This is possible because Petitclerc et al. (1985) demonstrated that exposition to L16 promotes a greater growth of parenchyma over adipose tissue in mammary gland of pre and post pubertal heifers, than in animals under eight h light. Again an interaction of IGF-1, IGFBP-5 and prolactin plays an important role in this effect of long photoperiod, in which IGF-1 promotes proliferation while inhibits apoptosis of mammary cells (Akers et al. 2005, Capuco et al. 2003), whereas IGFBP-5 sequesters IGF-1 in circulating blood and mammary gland preventing its binding to specific receptors in mammary tissues, thus avoiding the positive effects of IGF-1 described above. Besides, IGFBP-5 exerts a negative effect at mammary cells level by stimulating synthesis of proapoptotic molecules and by inhibiting actions of antiapoptotic agents in parenchymal and stromal cells (Flint et al. 2005); meanwhile prolactin inhibits these negative effects and enhances the positive IGF-1 actions by reducing IGFBP-5 synthesis in several tissues (Tonner et al. 1997). To provide further support to our speculation, information relative to mammary growth composition in heifers under L16 or natural photoperiod is currently under study. In a different line of thought, the higher percentage of heifers in estrus and the reduction of age to first estrus in response to exposure to 16 h light observed in this study provides evidence in support to a previous experiment carried out at 40° N in which exposure to L16 advanced puberty significantly in Holstein heifers (Rius et al. 2005). Therefore, the observed effects of L16 here, resemble those reported in latitudes ≥ 40 N relative to the initiation of reproductive activity despite cattle is considered non-seasonal breeders. The advancement of puberty in heifers exposed to 16 h light, documented in this study and in that of Rius et al. (2005), is a phenomenon understood partially, because long photoperiod despite reducing the deposition of adipose tissue, increases circulating levels of leptin in cattle (Dahl et al. 2000, Bernabucci et al. 2006), hormone thought to be a signal of somatic maturity and it was proposed by some authors as a triggering factor of puberty (Petitclerc an et al. 1983, Sejrsen 1994). However, there is evidence that leptin can also be produced by bovine mammary epithelial cells, phenomenon that is regulated at least partially by IGF-1 (Smith and Sheffield 2002). Thus, the increase of IGF-1 as a result of exposure of animals to supplementary light, could be one of the factors involved in the advancement of puberty, because it was observed that the highest luteinizing hormone (LH) response to exogenous kisspeptin, a secretagogue of gonadotrophin releasing hormone (GnRH) and through this of LH (Roa and Tena-Sempere 2010), was observed in prepubertal heifer calves that had the highest circulating levels of IGF-1 but not of leptin (Santos et al. 2014). The fact that the sustained increase in pulsatile secretion of GnRH and LH determines the first ovulation and estrous cyclicity in prepubertal females (Roa and Tena-Sempere 2010), makes possible the proposal of Santos et al. (2014), and Pinilla et al. (2012) that IGF-1 is an indicator of somatic maturity that trigger puberty, among other factors mentioned above such as leptin, adiponectin, insulin, ghrelin, glucocorticoids and estradiol (Roa and Tena-Sempere 2010, Pinilla et al. 2012, Tsang et al. 2014). Because in the present experiment exposure to L16 induced quantitative and qualitative changes in body growth as well as the advancement of puberty and mammary development in heifer calves, it is adequate to recognize that mammals possess systems, known as biological clocks, that detect changes in the external environment which also allows them to anticipate predictable environmental variations with an approximate duration of 24 h (circadian cycles): Adaptation of mammals with long biological cycles to these circadian cycles and the subsequent circannual cycles is considered as vital for their survival and adequate performance of their physiological functions (Lincoln et al. 2003).
The photoperiod effects imply the recognition of signals derived from variations in length of the diurnal segment of the day; these signals elicit seasonal changes of physiological and behavioral nature in animals (Goldman 2001). The light signals are detected by retinal photoreceptors that are not involved in the visual phenomena and via the retinohypothalamic tract, they eventually influence the pineal gland activity, whose main function consists in the melatonin release during the dark period (Goldman 2001, Tsang et al. 2014). Some neurons located in the suprachiasmatic nucleus, during the diurnal period release gamma-aminobutyric acid (GABA) which inhibits the sympathetic stimulation to the pineal gland, thus melatonin is not released; in contrast, during darkness GABA is not released and melatonin is secreted (Tsang et al., 2014). In mammals, melatonin plays a key role in modulating the physiological mechanisms that determine adaptation to seasonal changes (Lincoln et al. 2003). Scientific information indicates that this hormone acts mainly in the pars tuberalis of the adenohypophysis, Where three types of specific receptors for melatonin have been identified in mammals (Reppert et al. 1994, Browning et al. 2000), in cells named "calendar" which apparently decode the signals emitted by melatonin (Lincoln et al. 2003). This hormone, mainly acting in cells of the pars tuberalis of the anterior pituitary and to a lesser extent in the mid-basal and dorsolateral hypothalamus, exerts retrograde actions that activate or inactivate genes whose expression, or lack of it, determines the synthesis of the α and β chains of thyroid stimulating hormone (TSH), as well as actions of the deiodinase enzymes II and III, which in turn direct the transformation from prohormone thyroxine (T4) to the active hormone 3,5,3',5'-triiodothyronine (T3), or the degradation of T4 and T3 (Beltramo et al. 2014, Dardente et al. 2014), changes that participate in the regulation of reproductive functions in animals that are seasonal breeders (Barret et al. 2007, Beltramo et al. 2014, Dardente et al. 2014) and in some non-seasonal species (Beltramo et al. 2014). Melatonin also plays a role in the physiological modulation of the somatotropic and adrenocortical axes (Tsang et al. 2014). By a retrograde pathway (TSH / deiodinase), melatonin is involved in the regulation of reproduction by inhibiting indirectly the release of GnRH through the RFamide related peptides (Mason et al. 2010, Dardente et al. 2014), factors that depending of the time of the year when they are applied exogenously may also stimulate the GnRH release, apparently acting on neurons that secrete kisspeptin, a potent GnRH secretagogue (Beltramo et al. 2014). By the same retrograde path, melatonin is involved in the release of kisspeptin (Belgramo et al. 2014, Dardente et al. 2014) by a mechanism which is partially known but in the biochemical cascade it still remains elusive the factor that ultimately determines the melatonin indirect action on the kisspeptidergic cells (Beltramo et al. 2014). Some authors proposed that melatonin acting throughout the retrograde pathway, stimulates prolactin release by means of the TSH/deiodinase system, in which an intermediary factor is enhanced whose identity is not unanimously recognized but evidence has allowed to propose salsolinol, a dopamine derivative, as the putative prolactin secretagogue (Yaegashi et al. 2012), however, other researchers suggested an anterograde action of melatonin, exerting its positive effects on prolactin secretion by activating tuberalin (Graham et al. 2002).

CONCLUSIONS

In summary, relative to animals under natural photoperiod, exposure to L16 during 60 d to prepubertal Holstein heifers maintained in a subtropical area, promoted growth of skeletal muscle, pelvis and mammary gland, and induced the advancement of puberty but reduced adipose tissue growth; therefore we conclude that supplementary light promotes development of heifers with a higher productive potential and a lower risk of dystocia at first calving.

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