Maintenance of permanent sexual activity throughout the year in seasonal bucks using short photoperiodic cycles in open barns

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ABSTRACT

Seasonality of reproductive activity in rams and bucks is the major constraint in temperate and subtropical zones. Rapid alternation between 1 month of short days and 1 month of long days (**LD**) over three years in lightproof buildings eliminates this seasonality. We examined if this would also work in open barns, using only supplementary light. Over two years, one group of bucks (n = 7) was subjected to alternate 1 month of LD and 1 month of permanent light (LD–**LL**) and another group (n = 7) to alternate 1 month of LD and 1 month of natural light (LD–**NL**). A simultaneous control group, used for both experiments (**CG1**, n = 6; **CG2**, n = 6), remained under natural photoperiod. BW, testis weight (**TW**), plasma testosterone (**T**) and cortisol (**C**) were evaluated in all bucks. CG1 and CG2 bucks showed identical dramatic seasonal variations in BW (stable or decreasing in summer), TW (from 85 ± 12 g in February to 127 ± 7 g in July) and T (from 2.7 ± 1.2 ng/mL in January–April to 24.3 ± 3.2 ng/mL in June–October). By contrast, BW of LD–LL and LD–NL bucks increased regularly during the experiment. From 5 and 9 months after the experiment onset, LD–LL and LD–NL bucks, respectively, maintained constant TW of 115 ± 5 g until the experiment end. After the first 3 months <5 ng/mL, T of LD–LL bucks remained constant (5–10 ng/mL) until the experiment end. By contrast, T of LD–NL bucks showed four periods of low (<5 ng/mL) and two periods of high concentrations (18.1 ± 2.6 and 11.9 ± 3.4 ng/mL). Plasma C remained low (5–8 ng/mL) and did not change with group or light treatment. These results show for the first time in any seasonal photoperiodic species that it is possible to maintain the sexual activity of males all year round in open buildings using alternating periods of LD and LL. By contrast, return to NL instead of LL every other month does not prevent seasonality in T concentration. These results raise interesting questions about the photoperiodic control of neuroendocrine regulation of seasonal sexual activity and suggest that these treatments can be used to manage males in open barns in farms and in artificial insemination centres. (Spanish and French versions of the full text are available as Supplementary Materials S1 and S2).

Implications

Seasonal reproductive activity of rams and bucks results from complex neuroendocrine relationships under photoperiodic control that reduces their use during spring and summer. Previously, we showed that maintaining bucks under monthly alternations between long days and short days was able to eliminate this seasonality. However, this schedule requires the use of lightproof buildings. Here, using open barns, we show that monthly alternations between long days and permanent light produce the same result. This treatment can be proposed for farms and artificial insemination centres that do not have access to expensive lightproof buildings.

Introduction

The seasonality of sexual activity and its photoperiodic control in rams and bucks have been broadly described in temperate and subtropical zones (Pelletier et al., 1982; Poulton and Robinson, 1987; Delgadillo et al., 2002; Giriboni et al., 2017). Testis size, an indicator of spermatogenic processes occurring in the parenchy-

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mal tissue, testosterone secretion (**T**), an indicator of the activity of the interstitial tissue of the testis, and libido, a consequence of T action in the brain, vary dramatically throughout the year with low levels during sexual rest in spring and summer and high levels during the breeding season in autumn and winter (rams: Pelletier et al., 1982; bucks: Walkden-Brown et al., 1994; Giriboni et al., 2017). This phenomenon is a major drawback for farmers who want to perform autumn lambing and kidding, and for artificial insemination centres that have considerable difficulties in producing good-quality semen and in deep-freezing it when males are in sexual rest. The alternation between these opposites—rest *vs* activity—is governed by photoperiod that drives the neuroendocrine hypothalamic–pituitary axis (Gonadoliberin releasing hormone, LH and T). It was discovered that the very high levels of T in mid-winter were mainly responsible for the subsequent decrease in Gonadoliberin releasing hormone–LH activity by the negative feedback of T on LH pulsatile activity (Pelletier and Ortavant, 1975). Inhibiting the strong T increase by rapid alternations of long days (**LD**) and short days (**SD**) in an experimental lightproof building—what is referred to as "short photoperiodic cycles"—has allowed, for the first time in any photoperiodic species, maintenance of T at medium concentrations and LH and spermatogenic activities and libido at the same levels as those in the breeding season (Pelletier and Almeida, 1987; Almeida and Pelletier, 1988). These short photoperiodic cycles were applied in Alpine and Saanen bucks in an AI centre, and eliminated the seasonality of spermatogenic activity and enabled an increase of >70% of AI sperm doses compared with control bucks under natural light (Delgadillo et al., 1992; 1993). This scheme has been satisfactorily used for 20 years in French caprine AI centres and allows sperm production all year round.

However, these treatments are only applicable in expensive lightproof buildings in which SD during spring and summer can be delivered. A first successful attempt using open barns following the schedule of alternating LD and SD was carried out in Ile-de-France rams by alternating 1 month of LD with 1 month of a subcutaneous melatonin implant, which provided an SD-like signal. Rams maintained their testis weight (**TW**) at the level of the breeding season for more than 18 months. However, the difficulties encountered by repeatedly removing the melatonin implants after 1 month of insertion limit application of this regime in field conditions (Pelletier J., Chesneau D. and Chemineau P., unpublished results; Supplementary Fig. S1). Recently, it has been shown that providing permanent light (**LL**) after 2 months of LD was equivalent to the insertion of melatonin implants. In bucks and rams, this photoperiodic treatment improved T secretion and fertility (Delgadillo et al., 2016; Chesneau et al., 2017), thus suggesting the hypothesis that monthly alternations between LD and LL can be applied in open barns to eliminate the seasonality of sexual activity. This was the aim of our first experiment performed in Northern Mexico in Creole bucks, a seasonal photoperiodic local breed (Delgadillo et al., 2002). Because this area is located in a subtropical zone where the amplitude of photoperiodic changes is lower than in temperate zones, in a second experiment, we examined the hypothesis that the return to natural light (**NL**) after 1 month of LD might be perceived as SD. In addition, we tested the hypothesis that monthly alternations between LD and NL might be an efficient way to prevent sexual rest. Because the use of LL every other month in an LD–LL regime might be a potential source of stress in goats (Greenwood and Shutt, 1992), we measured plasma cortisol (**C**) concentrations in these bucks for comparison with control bucks.

Material and methods

General study conditions

The present study was conducted in the Laguna region of the state of Coahuila, Mexico (26°23′N, 104°47′W). The photoperiod in this region varies from 13 h 41 min at the summer solstice to 10 h 19 min at the winter solstice. The mean annual maximum and minimum temperatures vary from 37 °C in May–August to 6 °C in December–January. The Laguna region is characterised by a dry climate with an average annual rainfall of 266 mm (range, 163–504 mm), which generally occurs between June and September. Local male goats from the Laguna region, previously described as Creole goats, resulted from crosses between the Spanish Granadina, Murciana and Malagueña breeds, which were further crossed with Alpine, Saanen, Toggenbourg and Anglo-Nubian breeds in the last 50 years. In these male goats, sexual rest occurs from January to May (Delgadillo et al., 2002). In the present study, males were fed 2 kg of alfalfa hay per animal daily (9.6 MJ/kg, 18% CP per kg of DM; National Research Council, 2007) throughout the study, with free access to water and mineral salts.

General organisation of the experiments and groups

At the onset of each experiment, males were divided into groups balanced for BW and TW, and kept in different shaded open pens (10 × 5 m). Control males remained under NL throughout the study. Treated males were exposed to LD and LL by providing artificial light given from 0600 to 0800 and 1800 to 2200 (LD) and from 1800 to 0800 (LL). Artificial light was regulated by an electric clock, and light intensity was at least 300 lx, measured at the animals' eye level. Due to the difficulty in obtaining adult male goats and because of the high cost of maintaining adult and unproductive animals over more than two years, one single group of control males (27 months old at the study onset; n = 6; BW: 34 ± 1 kg; TW: 94 ± 4 g; mean ± SEM) was used for the whole experiment which lasted 32 months in total from 1 December 2017 to 31 July 2020 (Fig. 1). The control group was designated as **CG1** from 1 December 2017 to 30 November 2019 (experiment 1) and as **CG2** from 15 September 2018 to 31 July 2020 (experiment 2). This approach also allowed us to reduce the total number of experimental animals for ethical reasons (i.e., reduce, replace, refine).

In experiment 1, control males (CG1, n = 6) were compared with males of the experimental group LD–LL (27 months old at the study onset; n = 7; BW: 32 ± 1 kg; TW: 92 ± 2 g) that were subjected to alternations between 1 month of LD (16 h of light per day) and 1 month of LL over 24 months, starting in December, and combining natural and artificial light (Fig. 1). In experiment 2, control males (CG2, now 36 months old at the study onset, and n = 5 after one death in August 2018; BW: 52 ± 2 kg; TW: 111 ± 4 g) were compared with males of the same age that formed the experimental group LD–NL (36 months old at the study onset; n = 6; BW: 52 ± 1 kg; TW: 110 ± 6 g). LD–NL males were subjected to alternations between 1 month of LD and 1 month of NL over 23 months, starting in October (Fig. 1). The LD schedule was as in experiment 1.

Measurements

In experiments 1 and 2, BW of each male was determined once a month, and TW every two weeks by comparative palpation (Oldham et al., 1978). Plasma concentrations of T were determined

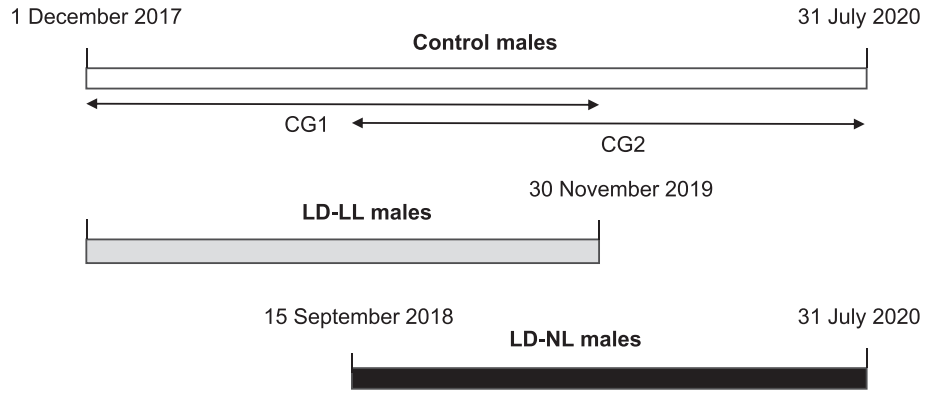

Fig. 1. Schematic representation of the experiments with Creole bucks from Northern Mexico. Over 24 months, animals were subjected in open barns to monthly alternations between 1 month of long days (LD) and 1 month of permanent light (LL; LD–LL males); or over 21 months, animals were subjected in open barns to monthly alternations between 1 month of LD and 1 month of natural light (NL; LD–NL males). Comparison was performed with control males (CG1 and CG2) maintained under NL throughout 32 months of the experiment.

every two weeks. All blood samples were collected by jugular venipuncture in 5-mL tubes containing 30 μL of heparin. Plasma was obtained after centrifugation at 2 500*g* for 20 min and stored at −20 °C until hormonal determination. The T concentration was determined using the immunoassays developed by our laboratory (Laboratoire Phénotypage et Endocrinologie, UMR PRC, INRAE, Nouzilly, France). We used a direct radioimmunoassay method for the first 17 samples of experiment 1, then a direct enzyme immunoassay method for the remaining samples of experiment 1 and for all samples of experiment 2. Both methods used an antibody against T that was previously characterised (Hochereau-De Reviers et al., 1990) and was the same as that used for the radioimmunoassay previously described (Delgadillo and Chemineau, 1992). Both methods were performed for each sample without an extraction process on 25 μl and 50 μl of plasma, respectively, for enzyme immunoassay and radioimmunoassay. Both methods included reference samples of known values at regular intervals during the assay, which allowed us to estimate the CV and were used as quality control (Delgadillo and Chemineau, 1992). The radioimmunoassay method was derived from Garnier et al. (1978) and Hochereau-De Reviers et al. (1990). The sensitivity was 0.3 ng/mL, and the intra-assay CV was 8.5%.

The enzyme immunoassay method is a competitive enzyme immunoassay in a 96-well plate. The plates were coated with T-specific antibody overnight at 4 °C. After washing with Tris-Tween 20, the plates were incubated with the sample together with the T alkaline phosphatase conjugate solution at 20 °C for 3 h. The plates were washed with Tris-Tween 20 and then incubated with pNpp. The absorbance was read at 405 nm with a plate reader. The sensitivity was 0.15 ng/mL, and the inter-assay CV was <5.8%. We validated the change from radioimmunoassay to enzyme immunoassay by assaying 62 goat plasma samples by both methods (Supplementary Fig. S2).

To detect any effect of stress due to light treatments, especially the LL schedule, we followed plasma C concentrations in a subset of the above plasma samples. In the control groups, we selected samples during the breeding season (four samples in each experiment) and during the rest season (two samples in experiment 1 and 4 in experiment 2). In the LD–LL group, we used selected samples taken in the middle of LD (three samples) and in the middle of LL (three samples). In the LD–NL group, we used selected samples taken in the middle of LD (four samples) and in the middle of NL (four samples). Samples of the control group and of the treated groups were taken on the same day.

Plasma C concentration was determined by competitive enzyme immunoassay in a 96-well plate on 25-μL aliquots of undiluted plasma, and all samples were analysed in the same assay. The plates were coated with goat anti-mouse IgG overnight at 4 °C. After a blocking step with Tris buffer, the plates were incubated with C-specific antibody at 24 °C for 1 h. Competition between the sample and the C alkaline conjugate solution was then initiated. The plates were washed and incubated with pNpp, which is detectable at 405 nm. The sensitivity was 4 ng/ml, and the intra-assay CV was <10%.

Statistics analysis

In experiments 1 and 2, BW, TW and T plasma concentrations were analysed using two-way repeated-measures ANOVA to detect differences between treatments. The model included the treatment (group), sampling time (weeks) and the interaction between these factors. We decided to use two-way ANOVA instead of an adjustment to a sinusoidal model that we had already used in bucks (Delgadillo et al., 1999) because the evolution of BW, TW and T plasma concentrations in LD–LL and LD–NL bucks clearly did not follow a sinusoidal trend as expected and as it occurred in CG1 and CG2 bucks. The independent *t* test was used to make posthoc comparisons when there were significant interactions. Because of the non-normal distribution of data as determined by the Lilliefors test, the C concentrations were compared using the Mann–Whitney *U* test to assess if at the different dates of sampling (i.e., under different photoperiodic conditions), there were differences between the control groups and the treated groups. The results are expressed as the mean ± SEM and differences were considered significant at the level of $P \leq 0.05$. All statistical analyses were performed using the System Statistics package (2009).

Results

BW

Control bucks of CG1 and CG2 groups showed identical substantial seasonal variations in BW, which were yearly bimodal, with growth from December to June and weight loss from July to November (Fig. 2a and b). CG1 group bucks regularly increased BW from December until July (52 ± 3 kg) when it decreased slightly (49 ± 2 kg) until December; it then increased until the following June of the second year (67 ± 2 kg) and decreased again until November (61 ± 2 kg). The same changes were observed in CG2 bucks, which started in October at a higher BW than CG1 bucks (52 ± 2 kg) and followed the same bimodal trend with growth from December (49 ± 2 kg) to June (71 ± 3 kg) and weight loss from July

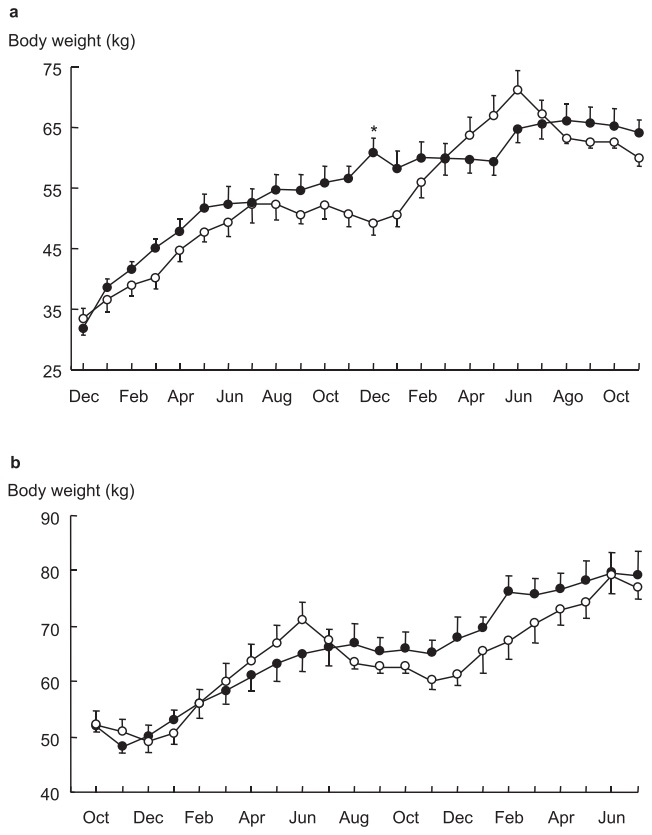

Fig. 2. BW (a; mean ± SEM) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of long days (LD) and 1 month of permanent light (LL; ●) over 24 months; these animals were compared with control bucks (CG1; ○) maintained under natural light (NL) throughout the experiment. BW (b; mean ± SEM) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of LD and 1 month of NL (●) over 21 months; these animals were compared with control bucks (CG2; ○) maintained under NL throughout the experiment. Month-to-month difference between groups was significant at $P < 0.05$*.

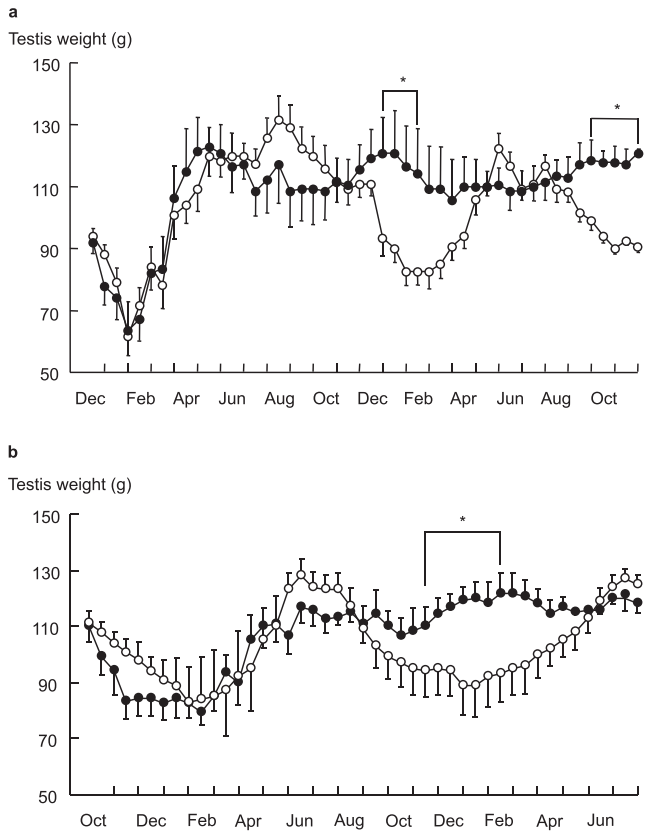

Fig. 3. Testis weight (a; mean ± SEM) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of long days (LD) and 1 month of permanent light (LL; ●) over 24 months; these animals were compared with control bucks (CG1; ○) maintained under natural light (NL) throughout the experiment. Testis weight (b; mean ± SEM) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of LD and 1 month of NL (●) over 21 months; these animals were compared with control bucks (CG2; ○) maintained under NL throughout the experiment. Month-to-month differences between groups were significant at $P < 0.05$*.

(67 ± 2 kg) to November (60 ± 1 kg). By contrast, LD–LL bucks did not show any seasonal variation in BW but rather showed a slow but permanent and regular increase during the whole experiment period, starting and ending at the same BW as CG1 bucks. The same observation was the case for LD–NL bucks, which showed a regular increase in BW that stabilised from August to November of the first year. We detected a significant effect of time on BW ($P < 0.001$) and a significant interaction between time and group ($P < 0.001$) in both experiments, indicating that BW varied differently over time between LD–LL and CG1 bucks on the one hand, and LD–NL and CG2 bucks on the other. Significant month-to-month differences were found only between CG1 and LD–LL bucks in December of the first year ($P < 0.05$) (Fig. 2a and b).

Testis weight

CG1 and CG2 bucks showed identical dramatic seasonal variations in TW with minima in February (CG1: 62 ± 7 g and 83 ± 13 g; CG2: 84 ± 15 g and 93 ± 10 g) and maxima in July (CG1: 126 ± 8 g and 117 ± 7 g; CG2: 123 ± 6 g and 127 ± 4 g) in the two years, respectively (Fig. 3a and b). These variations were identical in both years. By contrast, 5 months after the experiment onset in LD–LL bucks and after 9 months in LD–NL bucks, we observed that their TW remained constant (115 ± 5 g) until the experiment end. It appears that LD–LL bucks had TW that stabilised earlier around the above mean than the LD–NL bucks, which require more time to stabilise TW. The interval between the onset of photoperiodic treatments and maximum testicular weight appears to be shorter in the LD–LL group (159 ± 13 days) than in the LD–NL group (275 ± 10 days). We detected a significant effect of time on TW ($P < 0.001$) and a significant interaction between time and group ($P < 0.001$) in both experiments, indicating that TW varied differently over time between LD–LL and CG1 bucks on the one hand and LD–NL and CG2 bucks on the other. Significant month-to-month differences appeared between CG1 and LD–LL bucks in January–February and October–November of the second year, and between CG2 and LD–NL bucks in December–March of the second year ($P < 0.05$) (Fig. 3a and b).

Testosterone concentrations

CG1 and CG2 bucks showed identical dramatic seasonal variations in T from <5 ng/mL (2.7 ± 1.2 ng/mL) in January–April to 25 ng/mL (24.3 ± 3.2 ng/mL) in June–October (Figs. 4a and 5a). These variations were identical in both years and both experiments. By contrast, 4 months after the experiment onset, LD–LL bucks, despite less month-to-month variation, maintained medium T concentrations of 5–14 ng/mL (8.6 ± 2.1 ng/mL) until the experiment end. The situation was very different in LD–NL bucks, which showed dramatic variations over the course of the experiment, with two periods of low T concentrations <5 ng/mL in December–June (3.1 ± 1.2 ng/mL) of the first year, in December–

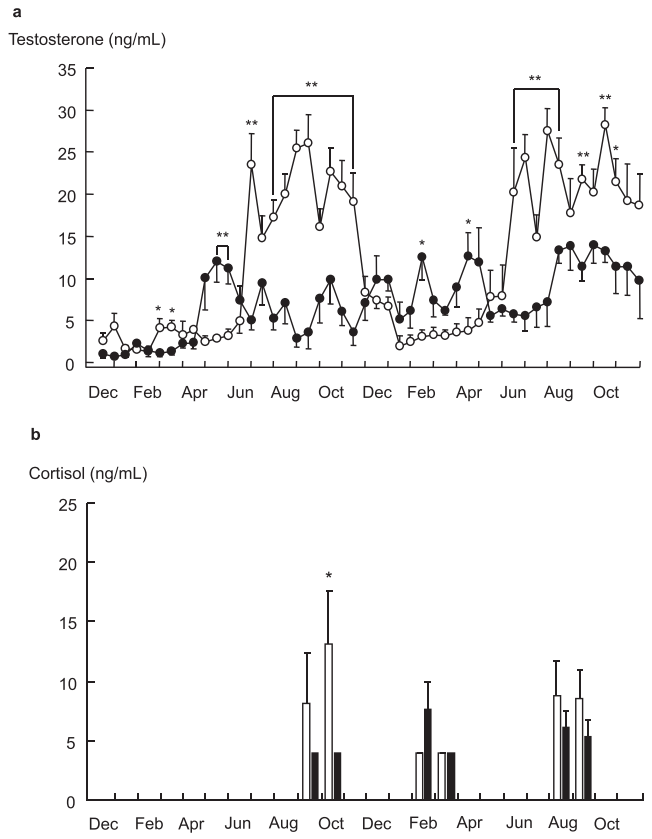

Fig. 4. Plasma testosterone (mean ± SEM) (a; ●) and cortisol (mean ± SEM) (b; ■) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of long days (LD) and 1 month of permanent light (LL) over 24 months. Comparison was performed with control bucks (CG1; ○, □, respectively) maintained under natural light throughout the experiment. Month-to-month differences between groups were significant at $P < 0.05$* or $P < 0.01$**.

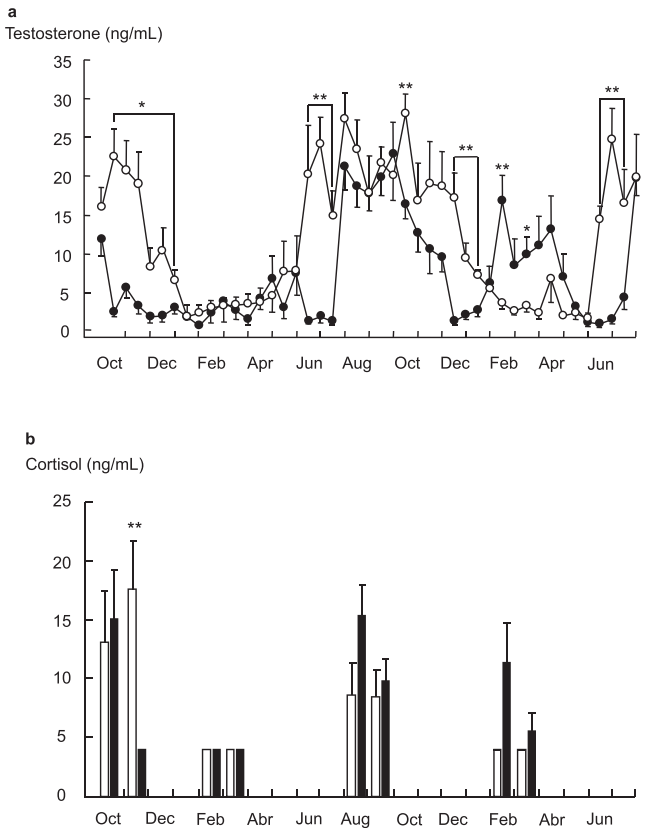

Fig. 5. Plasma testosterone (mean ± SEM) (a; ●) and cortisol (mean ± SEM) (b; ■) of Creole bucks from Northern Mexico subjected in open barns to monthly alternations between 1 month of long days (LD) and 1 month of natural light (NL) over 21 months. Comparison was performed with control bucks (CG2; ○, □, respectively) maintained under NL throughout the experiment. Month-to-month differences between groups were significant at $P < 0.05$* or $P < 0.01$**.

January (1.5 ± 0.6 ng/mL) and May–July (2.3 ± 0.9 ng/mL) of the second year, and two periods of high concentrations in July–November (16.7 ± 2.6 ng/mL) of the first year and in January–April (10.5 ± 3.1 ng/mL) of the second year. LD–NL bucks were not able to maintain a medium T concentration as did LD–LL bucks. We detected a significant effect of time on T concentration ($P < 0.001$) and a significant interaction between time and group ($P < 0.001$) in both experiments, indicating that T concentrations varied differently over time between LD–LL and CG1 bucks on the one hand and LD–NL and CG2 bucks on the other. Two by two comparison showed a high number of significant month-to-month differences between control and treated groups over the course of the experiments (Figs. 4a and 5a).

Cortisol concentrations

Plasma C concentrations remained low during the whole experiment (Figs. 4b and 5b). LD–LL and LD–NL bucks showed similar mean plasma C concentrations to those of the CG1 and CG2 groups (5.2 ± 1.3 and 8.7 ± 2.8 *vs* 7.7 ± 3.0 and 8.0 ± 3.1 ng/mL of plasma, respectively). Comparing photoperiodic conditions, we detected only two significant differences: in experiment 1, plasma C concentration of CG1 bucks during the breeding season was significantly higher than that of LD–LL bucks subjected to LD (13.2 ± 4.4 *vs* 4.0 ± 0.0 ng/mL; $P = 0.02$) (Fig. 4b); in experiment 2, plasma C concentration of CG2 bucks during the breeding season was significantly higher than that of LD–NL bucks subjected to NL (17.7 ± 4.2 *vs* 4.0 ± 0.0 ng/mL; $P = 0.01$) (Fig. 5b). Other variables did not differ significantly.

Discussion

The results obtained in LD–LL bucks clearly show that maintaining a permanent high TW and medium plasma T concentration is possible in open barns while animals still perceived NL. To our knowledge, this is the first report of such a result obtained in any seasonal and photoperiodic animal species, even model ones. This result is of interest from a scientific perspective as we were able to completely bypass the strong inhibition of photoperiod on sexual activity occurring every year. In addition, it is interesting from a practical point of view for farmers and AI centres, which can now apply this treatment in open barns, at low cost and with minimal technicality.

By contrast, LD–NL bucks did not experience the same elimination of seasonal T variation and did not show the same evolution of TW over the months of the experiment as did LD–LL bucks. The variation in mean T in both groups of light-treated bucks over the course of the experiments is also notable. Maintaining a medium and stable level of T in LD–LL bucks (5–15 ng/mL of plasma) compared with CG1, CG2 and LD–NL bucks provides support to the hypothesis proposed by Pelletier and Almeida (1987) and Almeida and Pelletier (1988) in rams and confirmed by Delgadillo and Chemineau (1992) in bucks. That is, by preventing very high T concentrations in the blood, the strong negative feedback of T on the hypothalamic–pituitary axis was prevented as

well as the reduction in Gonadoliberin-Releasing Hormone–LH activity, which probably occurred in November–December in our CG1 and CG2 bucks.

These results also confirm that LL over 1 month and following 1 month of LD gave similar results of TW and T secretion as did SD and/or melatonin implants, as reported by Delgadillo et al. (2016) in bucks and Chesneau et al. (2017) in rams. This likely means that the real signal perceived by animals after LD is more the absence of signal or the cessation of LD rather than the effect of SD or melatonin, which is responsible for triggering sexual activity in these males. This facilitates the use of the proposed alternations, which require only minimal equipment, whereas difficulties have been encountered with the successive use of 1 month melatonin implants.

By contrast, the inefficiency of the second light scheme tested here, the LD–NL treatment, is disappointing and does not support our initial hypothesis that the return to NL after 1 month of LD would be perceived as SD able to stimulate the hypothalamic–pituitary axis. In spite of the low amplitude of photoperiod in this subtropical area (∼10 h and ∼14 h of light at the winter and summer solstices, respectively), the LD–NL schedule does not allow—most likely in the spring—the perception of SD by these Creole bucks. Rather, the animals probably continue to perceive LD at that time of the year. From comparison of the T concentrations of LD–NL bucks (Fig. 5a) with those of LD–LL bucks (Fig. 4a), it is clear that the former failed to benefit from the short photoperiodic cycles, and in June–July of the two-year experiment, they behaved as CG2 bucks. It is possible that after a 1 month delay, they experienced a very strong increase in T over the following months. This strong increase is likely responsible for the subsequent decrease later in October, as in CG2 bucks.

In our study, plasma C concentrations remained low in all bucks compared with other results obtained in Mexico (Ortiz-de-Montellano et al., 2007; Sánchez-Dávila et al., 2018), and only two points at which bucks were subjected to different photoperiods were significant. In these two cases, the control bucks showed higher plasma C concentrations than light-treated bucks. This result indicates that these two light schedules did not exert any detectable stress on our animals and can therefore be used satisfactorily without any negative impact on the welfare of animals.

In conclusion, we believe that this new LD–LL treatment, which is cheap and easily applicable in open barns, could be an efficient way to eliminate the seasonal variations of sexual activity that represent one of the major constraints for the management of small ruminant males, especially in artificial insemination centres that want to produce semen of high quality all year round.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101041.

Ethics approval

The experimental procedures were in accordance with the official Mexican rules governing the technical specifications for the production, care, and use of laboratory animals (Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio, 2001).

Data and model availability statement

None of the data were deposited in an official repository but are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the first and last authors used Google Translate in order to translate the first version of this article in Spanish and French. After using this service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication. Spanish and French versions are available as Supplementary Materials 1 and 2.

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Author contributions

J.A. Delgadillo: Conceptualisation, Methodology, Resources, Writing-Original draft preparation, Writing-Reviewing & Editing. **L.A. Espinoza-Flores**, **D. López-Magaña**, **H. Hernández**: Conceptualisation, Investigation, Formal analysis, Writing-Reviewing & Editing. **A.L. Lainé**: Testosterone and Cortisol assays, Writing-Reviewing & Editing. **M. Keller**, **D. Chesneau**, **P. Chemineau**: Conceptualisation, Methodology, Writing-Original draft preparation, Writing-Reviewing & Editing.

Declaration of interest

None.

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