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### Nutritional supplementation improves the sexual response of bucks exposed to long days in semi-extensive management and their ability to stimulate reproduction in goats

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#### ABSTRACT

In confined management systems, well-nourished bucks rendered sexually active by exposure to long days are efficient in fertilizing out-of-season goats. However, underfeeding is common in semi-extensive management systems and may reduce the reproductive efficiency of bucks. The objective of the present study was to determine whether nutritional supplementation improved the sexual activity of bucks submitted to long days in semi-extensive management systems and their ability to stimulate the reproduction of goats in semi-extensive or confined conditions. In experiment 1, three groups of bucks were placed in different flocks and grazed daily with females for 7 h. Each day after grazing, males were separated from females and moved into open pens. One group did not receive any treatment (control group;  $n = 6$ ). Two other groups were submitted to artificially long days from 15 November to 15 January. From 16 January, one group did not receive nutritional supplementation (long-day group;  $n = 5$ ), whereas bucks from the other group each received 600 g of a commercial concentrate (long-day + supplementation group;  $n = 5$ ). The fourth group was kept in confined conditions, exposed to long days and fed alfalfa hay (long-day confined group;  $n = 6$ ). On 26 March, anovulatory goats from other flocks were assigned to four groups ( $n = 27$  each) and confined separately in open pens. Three bucks of each group were housed with the females. Pregnancy rates were greater in the goats housed with the long-day group than those housed with the control group ( $P < 0.01$ ). However, pregnancy rates did not differ between the long-day confined group (89%) and long-day + supplementation group (70%;  $P = 0.09$ ), but these rates were greater than those from the long-day (37%) and control groups (0%;  $P < 0.05$ ). In experiment 2, two groups of males ( $n = 3$  each) were incorporated into two flocks under semi-extensive management and grazed daily with females for 7 h. One group of males did not receive any treatment (control group). The other group was submitted to long days and nutritional supplementation as in experiment 1 (long-day + supplementation group). Males remained with females during the whole study. The pregnancy rate was greater in the goats joined by males of the long-day + supplementation group (78%) than in those from the control group (0%;  $P < 0.001$ ). We conclude that long days and nutritional supplementation improve the ability of bucks kept in semi-extensive management to stimulate reproduction of out-of-season goats in confined or semi-extensive management systems.

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#### Implications

These results indicated that providing increased nutrition to light-exposed bucks improved out-of-season reproductive success compared to exposure to artificially long days alone. No differences between

supplementation and confinement management systems were evident in the current study.

#### Introduction

Reproductive seasonality is a trait of many breeds of goats from subtropical latitudes. In male goats from these latitudes, sexual rest lasts from January to June, whereas in females, the anestrus season lasts from February to August in the Northern Hemisphere (Delgadillo

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et al., 1999; Duarte et al., 2008). This reproductive seasonality is mainly timed by annual photoperiodic changes (Delgadillo et al., 2004; Duarte et al., 2010). Nonetheless, the reproductive seasonality can be modified by other environmental cues, such as the presence of conspecifics, particularly the “male effect” (Walkden-Brown et al., 1993; Chemineau et al., 2006). Indeed, the introduction of a male into a group of seasonally anestrus goats can stimulate ovulatory activity in the few days the males are introduced (Shelton, 1960; Pellicer-Rubio et al., 2007; Bedos et al., 2014). In strongly seasonal breeds, the response of the females housed with males is modified by the intensity of their sexual behavior. Hence, males that have been rendered sexually active by exposure to artificially long days and that are displaying intense sexual behavior during the rest season are able to induce more seasonally anestrus goats to ovulate than control males displaying weak sexual behavior (Delgadillo et al., 2002; Chasles et al., 2016; Zarazaga et al., 2019). Interestingly, the continuous presence of sexually active males stimulates most females to ovulate during seasonal anestrus rather than the continuous presence of control males (Delgadillo et al., 2015). Together, these results indicate that the introduction or the continuous presence of sexually active males stimulates ovulation in seasonally anestrus goats and that the intensity of their sexual behavior plays an important factor in the response.

The nutritional conditions of male goats dramatically modify the response to long-day treatment and the ability to stimulate ovulation in seasonally anestrus goats. Indeed, undernourishment dramatically reduced the scrotal circumference and the intensity of sexual behavior of males submitted to long-day treatment (Delgadillo et al., 2020). In addition, pregnancy rates were significantly lower in females joined by undernourished males kept under a natural photoperiod than in those joined by well-nourished males (Walkden-Brown et al., 1993). Therefore, undernutrition reduced the ability of male goats to stimulate sexual and reproductive activities in seasonally anestrus goats. In subtropical latitudes, most goats are managed in semi-extensive management systems, in which male and female goats are together year-round, walk 5 to 8 km daily, and graze for approximately 8 h per day, eating only the available vegetation without supplementary feed (Duarte et al., 2008; Andrade-Esparza et al., 2018). During the dry season, food availability decreases markedly, leading to undernutrition during sexual rest (Delgadillo and Martin, 2015). It is likely that the characteristics of the semi-extensive management systems might modify the response of males submitted to long days and then their ability to stimulate reproduction in female goats during seasonal anestrus. Therefore, the objectives of the present study, which, to our knowledge, have never been tested before, were to determine whether nutritional supplementation improved the sexual activity of bucks exposed to artificially long days in a semi-extensive management system and whether these supplemented males were able to stimulate the reproduction of goats in semi-extensive or confined management systems.

## Material and methods

### General conditions of study

The study was performed in the Laguna region of the state of Coahuila, Mexico (latitude 26°23' N; longitude, 104°47' W). The photoperiod in this region varies from 1341 h at the summer solstice to 1019 h at the winter solstice, whereas the mean annual maximum and minimum temperatures vary from 37 °C between May and August to 6 °C between December and January. The Laguna region is characterized by a dry climate with an average annual rainfall of 266 mm (range: 163 to 504 mm), which generally occurs between June and September (Duarte et al., 2008). Local animals from the Laguna region, which have previously been described as Creole goats, were used (Duarte et al., 2008).

### Experiment 1

#### Nutritional and photoperiod treatments of males

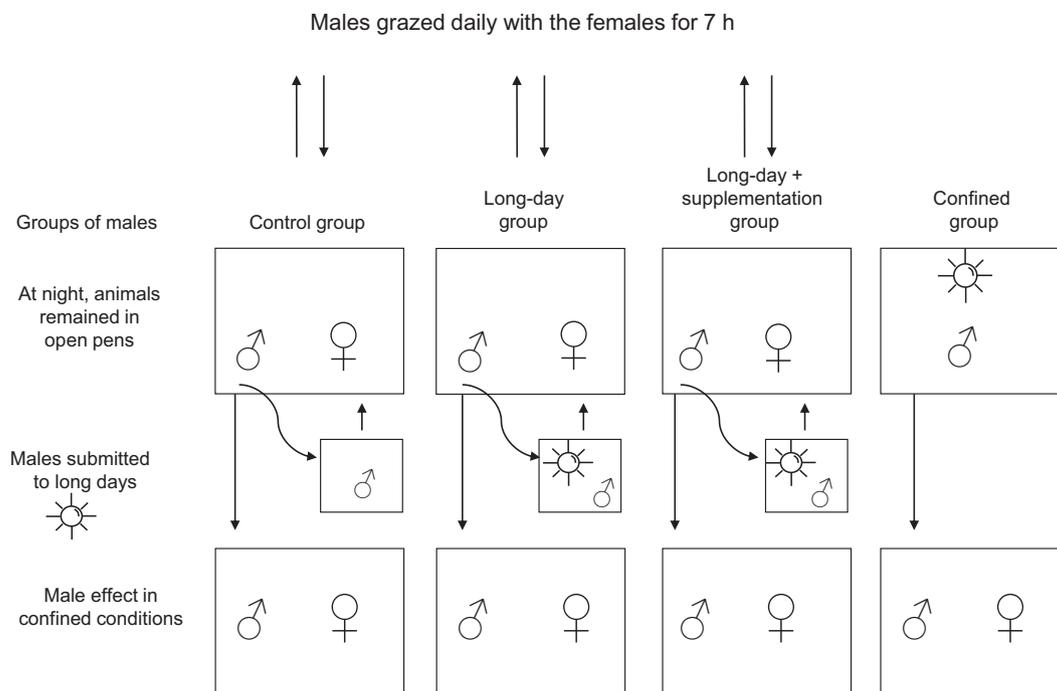
Sexually experienced male goats were used. The rationale for choosing the number of males per group (5 or 6) was based on (a) the expected differences in the results of the measurements of BW, body condition (BC), testicular weight, plasma testosterone, and sexual behavior; (b) the need for a security margin to be able to randomly select three males out of five or six to be used for the buck effect; and (c) the availability of experimental animals. From 1 November, males were assigned to four groups balanced for BW, BC, and testicular weight. Males of three groups were placed in three different flocks with 60–70 multiparous females each and grazed daily with the females on different routes from 1000 h to 1700 h. Each day after grazing, the males from each flock were separated from the females and moved into open pens (6 × 4 m each). One group did not receive any treatment and formed the control group ( $n = 6$ ; BW:  $59 \pm 5$  kg; BC:  $2.2 \pm 0.1$ ; testicular weight:  $99 \pm 4$  g; mean  $\pm$  SEM). The two other groups were exposed to artificially long days (16 h of light per day) from 15 November to 15 January followed by natural variations in the photoperiod. Artificial light was provided from 0600 h to 0800 h and from 1800 h to 2200 h. The pens of males were at least 50 m from those of does, which did not receive the photoperiod treatment. From 16 January, the bucks from one photoperiod-treated group did not receive any nutritional supplementation and formed the long-day group ( $n = 5$ ; BW:  $58 \pm 10$  kg; BC:  $2.2 \pm 0.1$ ; testicular weight:  $99 \pm 6$  g). Bucks from the other group were each supplemented with 600 g of commercial concentrate (1.7 Mcal/kg, 14% CP per kg of DM) before grazing and formed the long-day + supplementation group ( $n = 5$ ; BW:  $59 \pm 8$  kg; BC:  $2.2 \pm 0.1$ ; testicular weight:  $103 \pm 4$  g). During grazing, all males were fitted with an apron to prevent mating with females. The fourth group of bucks was kept in confined conditions 20 km away from the other three groups of bucks. These males were isolated from females and exposed to artificially long days as described above and formed the long-day confined group ( $n = 6$ ; BW  $58 \pm 9$  kg; BC:  $2.1 \pm 0.1$ ; testicular weight:  $98 \pm 4$  g). They were fed 2 kg of alfalfa hay per animal daily (2.3 Mcal/kg, 18% CP per kg of DM; National Research Council (NRC), 2007). The experimental protocol is shown in Fig. 1.

#### Conditions of females

There were three different flocks of females from those where males were placed during the photoperiod treatment. Females were multiparous and had delivered between October and December and were hand-milked once per day during the study. From October until 25 March, the females were isolated from the males (see male effect section). The anovulatory state of the goats was determined by transrectal ultrasonographies performed on 3, 13, and 23 March using an Aloka SSD-500 device (Co., LTD, Tokyo, Japan) connected to a transrectal 7.5-MHz linear probe (Simões et al., 2007). Females without corpora lutea in the three observations were considered to be anovulatory. On 23 March, the anovulatory females were assigned to four groups ( $n = 27$  each) balanced based on their BC ( $1.4 \pm 0.1$ , all groups) and confined separately in shaded open pens (20 × 20 m). The rationale for the number of females per group was based on (a) the expected differences in frequencies among groups using a  $\chi^2$  analysis (see below) and (b) availability of experimental animals. Females were fed 2 kg of alfalfa hay daily (2.3 Mcal/kg, 18% CP per kg of DM) and 100 g of commercial concentrate (1.7 Mcal/kg, 14% CP per kg of DM; National Research Council (NRC), 2007).

#### The male effect

On 26 March (day 0), the four groups of goats were joined by three males taken at random from each group of males. The males remained in contact with females for 15 consecutive days, as reported previously (Fitz-Rodríguez et al., 2009; Araya et al., 2016). Each group of goats was divided into three subgroups by a wooden barrier, allowing visual,



**Fig. 1.** Experimental design of experiment 1 showing movements (arrows) in four groups of male goats. Three groups were kept in a semi-extensive management system and grazed daily with females from 1000 h to 1700 h. At night, males were moved to another open pen. One group did not receive any treatment (control group). Two other groups were subjected to artificially long days (16 h of light by day) between 15 November and 15 January, followed by natural variations in the photoperiod. After the end of long days, one group did not receive any nutritional supplementation (long-day group), whereas bucks from the other group were individually supplemented with 600 g of a commercial concentrate (long-day + supplementation group). The fourth group was kept in confined conditions and exposed to the same artificially long days as the other two groups and fed 2 kg of alfalfa hay per animal daily (long-day confined group). On 26 March, four groups of goats ( $n = 27$  each) were joined by three males taken at random from each group of males.

auditory, and nose-to-nose contact among animals; this division was made to prevent fighting and accidents among males. Hence, each buck was in contact with 9 females, and males were rotated daily in their respective groups to avoid individual effects. During the test of the male effect, males were fed to maintain their BW (National Research Council (NRC), 2007).

#### Measurements

BWs were determined every two weeks between November and March before the distribution of nutritional supplementation, which was measuring using an electronic balance with a capacity of 250 kg and a precision of 0.05 kg (Torrey, Nuevo León, México).

Body conditions were determined every two weeks between November and March using a scale ranging from 1 (very lean) to 4 (obese), which was based on lumbar palpation (Walkden-Brown et al., 1997).

Testicular weights were determined at the beginning of November and then every two weeks between January and March by comparative palpation with an orchidometer (Oldham et al., 1978).

Plasma concentrations of testosterone were determined every two weeks between December and March. All blood samples were collected by jugular venipuncture in 5-mL tubes containing 30  $\mu$ L of heparin. Plasma was obtained after centrifugation at 2500 g for 20 min and stored at  $-20^{\circ}\text{C}$  until hormonal determination. The plasma concentrations of testosterone were determined using a direct RIA method 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%.

Sexual behavior displayed by bucks in the presence of females was monitored from 0830 h to 0900 h during the first three days after their introduction into the female groups. Trained observers followed the bucks individually and recorded the number of nudging events

(Bedos et al., 2016). Pregnancy rates were determined by the presence of embryos according to transrectal ultrasonography 40 days after the introduction of males in all groups (González de Bulnes et al., 1998).

#### Experiment 2

##### Nutritional and photoperiod treatments of males

Sexually experienced male goats were used. Males were assigned to two groups balanced for BW. From 1 November, the males of each group were placed into two different flocks with 90–100 females each and grazed daily with the females on different routes from 1000 h to 1700 h. Each day after grazing, the males from each flock were separated from the females and moved into open pens (6  $\times$  4 m each). One group did not receive any treatment and formed the control group ( $n = 3$ ; BW:  $56 \pm 5$  kg). The bucks in the supplemented group were exposed to artificially long days from 15 November to 15 January and were individually supplemented with 600 g of commercial concentrate from 16 January until the study ended on 15 May; they formed the long-day + supplementation group ( $n = 3$ ;  $58 \pm 6$  kg). From 1 November, the bucks remained together with females of their respective flocks during the entire study. From 1 November to 11 March, all males were fitted with an apron to prevent them from mating with females and removed on 12 March.

##### Preparation of females and measurements

Females were multiparous and had delivered in September and October, and they were hand-milked once per day during the study. The anovulatory state of goats was determined on 24 February, 3 and 10 March as described in experiment 1. On March 10, the females from each flock ( $n = 70$  each) were balanced by BC ( $1.5 \pm 0.1$ , both flocks). The rationale for choosing the number of females per group was based on (a) the expected differences in frequencies among groups using a

$\chi^2$  analysis and (b) the availability of experimental animals. Pregnancy rates were determined by transrectal ultrasonography on 15 May as described in experiment 1.

### Statistics analysis

In experiment 1, the BW, testicular weight, and plasma concentrations of testosterone were analyzed using 2-way repeated-measures ANOVA to detect differences among treatments. The model included the treatment (group), sampling time (weeks), and the interaction between these factors, followed by Fisher's exact test for  $2 \times 2$  individual point comparisons. Because of the ordinal nature of the data for BC, this variable was analyzed for a general time effect by the Friedman test and by Kruskal-Wallis followed by the Mann-Whitney U test for comparison between the 2 groups at a given time. The male sexual behavior was analyzed using the  $\chi^2$  test for goodness of fit, and a random distribution of 50% in each group was considered when the four groups of males were compared. The proportion of females that ovulated and the pregnancy rates were compared between groups using the  $\chi^2$  test. When there was a significant difference, comparisons between groups were made using Fisher's exact test. In experiment 2, the pregnancy rates were compared using the  $\chi^2$  test. The data are expressed as the mean  $\pm$  SEM, and differences were considered significant at the level of  $P \leq 0.05$ . All statistical analyses were performed using the [System Statistics \(2009\)](#). The results are expressed as the mean  $\pm$  SEM.

## Results

### Experiment 1

#### Body weights

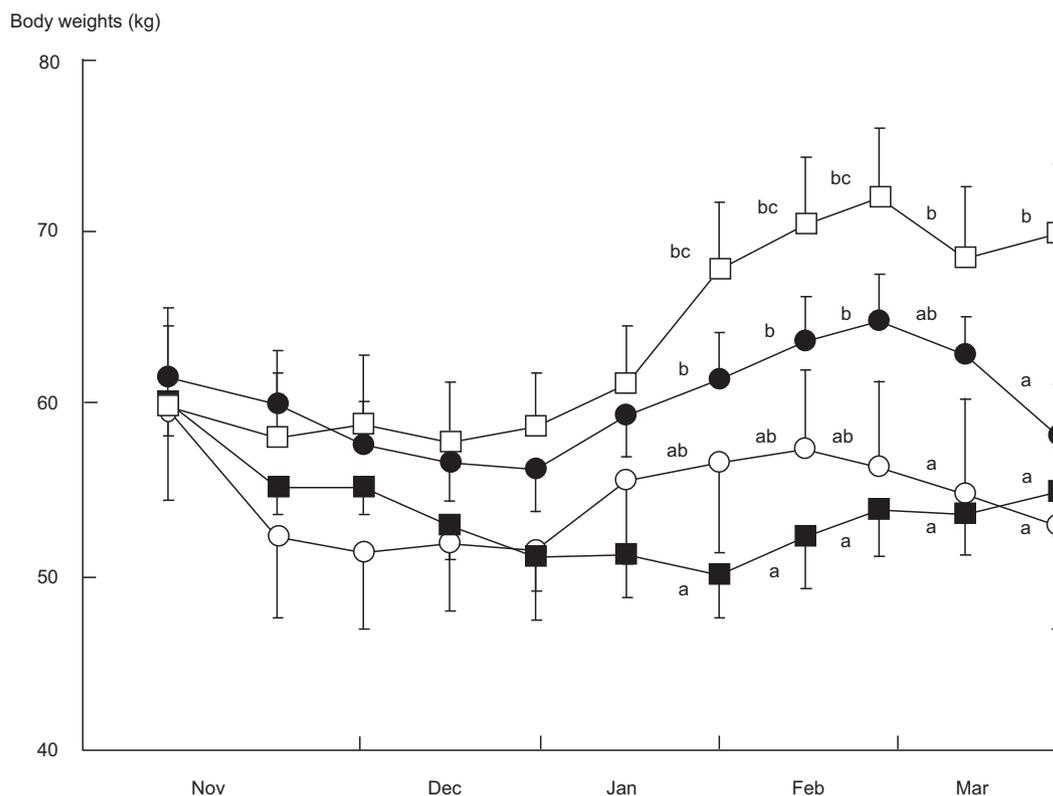
A treatment by time interaction was detected for BW ( $P < 0.001$ ), necessitating examination of the treatment effects within time (Fig. 2). Body weights did not differ among groups from 1 November to 15 January ( $P > 0.05$ ). Thereafter, the BW increased from 30 January in the three photoperiod-treated groups. In the control group, the increase in BW occurred one month later. On 31 March, the BW of the confined group was much greater than that of both the long-day and control groups ( $P < 0.05$ ).

#### Body conditions

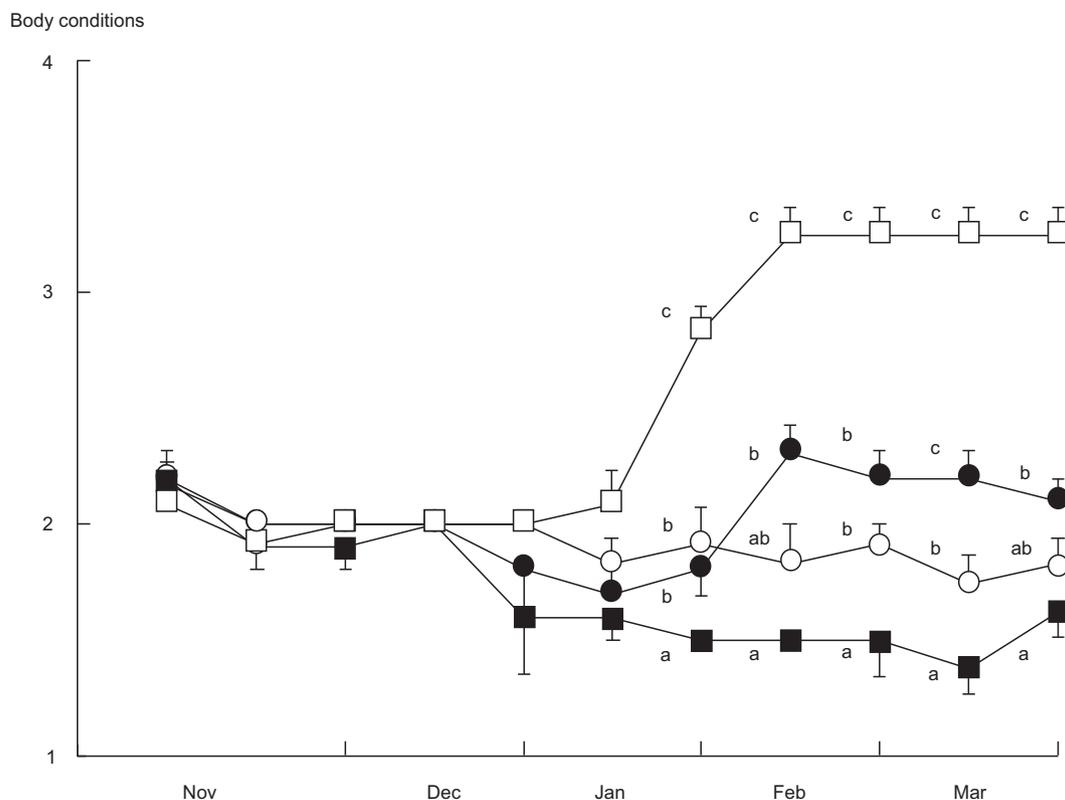
A treatment by time interaction was detected for BC ( $P < 0.001$ ; Fig. 3). Body conditions did not differ among groups in November and December ( $P > 0.05$ ). Thereafter, BC increased dramatically in the long-day confined group, and the values of this variable were greater than those of the other three groups until the end of study ( $P < 0.01$ ). In the long-day and long-day + supplementation groups, BC did not differ from January to March ( $P > 0.05$ ), but the BC of these two groups was greater than that of the control group between 30 January and 15 March ( $P > 0.05$ ).

#### Testicular weights

A treatment by time interaction was detected for testicular weights ( $P < 0.001$ ; Fig. 4). Testicular weights did not differ among groups on



**Fig. 2.** Body weights (mean  $\pm$  SEM) in four groups of male goats. Three groups were kept in a semi-extensive management system and grazed daily from 1000 h to 1700 h. One group did not receive any treatment (control group; ■). Two other groups were subjected to artificially long days (16 h of light by day) between 15 November and 15 January, followed by natural variations in the photoperiod. After the end of long days, one group did not receive any nutritional supplementation (long-day group; ○), whereas bucks from the other group were individually supplemented with 600 g of a commercial concentrate (long-day + supplementation group; ●). The fourth group was kept in confined conditions, exposed to the same artificially long days as the other two groups and fed 2 kg of alfalfa hay per animal daily (long-day confined group; □). Body weights were determined every two weeks. Different letters indicate significant differences among groups ( $P < 0.05$ ).



**Fig. 3.** Body conditions (mean  $\pm$  SEM) in four groups of male goats. Three groups were kept in semi-extensive management and grazed daily from 1000 h to 1700 h. One group did not receive any treatment (control group; ■). Two other groups were subjected to artificially long days (16 h of light by day) between 15 November and 15 January, followed by natural variations in the photoperiod. After the end of long days, one group did not receive any nutritional supplementation (long-day group; ○), whereas bucks from the other group were individually supplemented with 600 g of a commercial concentrate (long-day + supplementation group; ●). The fourth group was kept in confined conditions, exposed to the same artificially long days as the other two groups and fed 2 kg of alfalfa hay per animal daily (long-day confined group; □). Body conditions were determined every two weeks. Different letters indicate significant differences among groups ( $P < 0.05$ ).

1 November and 15 January ( $P > 0.05$ ). In the photoperiod-treated groups, testicular weights increased progressively between 30 January and 30 March. The testicular weights from the long-day confined group were greater than those of the long-day group between 29 February and 30 March ( $P < 0.05$ ), but they did not differ from those of the long-day + supplementation group ( $P > 0.05$ ). On 30 March, the testicular weights were greater in the three photoperiod-treated groups than in the control group ( $P < 0.05$ ).

#### Plasma concentrations of testosterone

A treatment by time interaction was detected for plasma concentrations of testosterone ( $P < 0.001$ ; Fig. 5). Testosterone concentrations were low and did not differ among groups between December and February. In March, the testosterone concentrations increased dramatically in the three photoperiod-treated groups, but these concentrations did not differ among groups ( $P > 0.05$ ). Hence, in March, the plasma testosterone concentrations in the photoperiod-treated bucks were greater than those registered in the control group ( $P < 0.05$ ).

#### Sexual behavior of bucks

The number of nudging events displayed by bucks differed among groups ( $P < 0.001$ ), and the long-day confined group displayed more nudging than the other three groups ( $P < 0.001$ ; Table 1).

#### Ovulatory response and pregnancy rates

The proportion of females that ovulated after joining by males differed among groups ( $P < 0.01$ ). Indeed, all females joined by photoperiod-treated males ovulated, whereas none ovulated when joined by the control males ( $P < 0.01$ ; Table 2). Pregnancy rates also

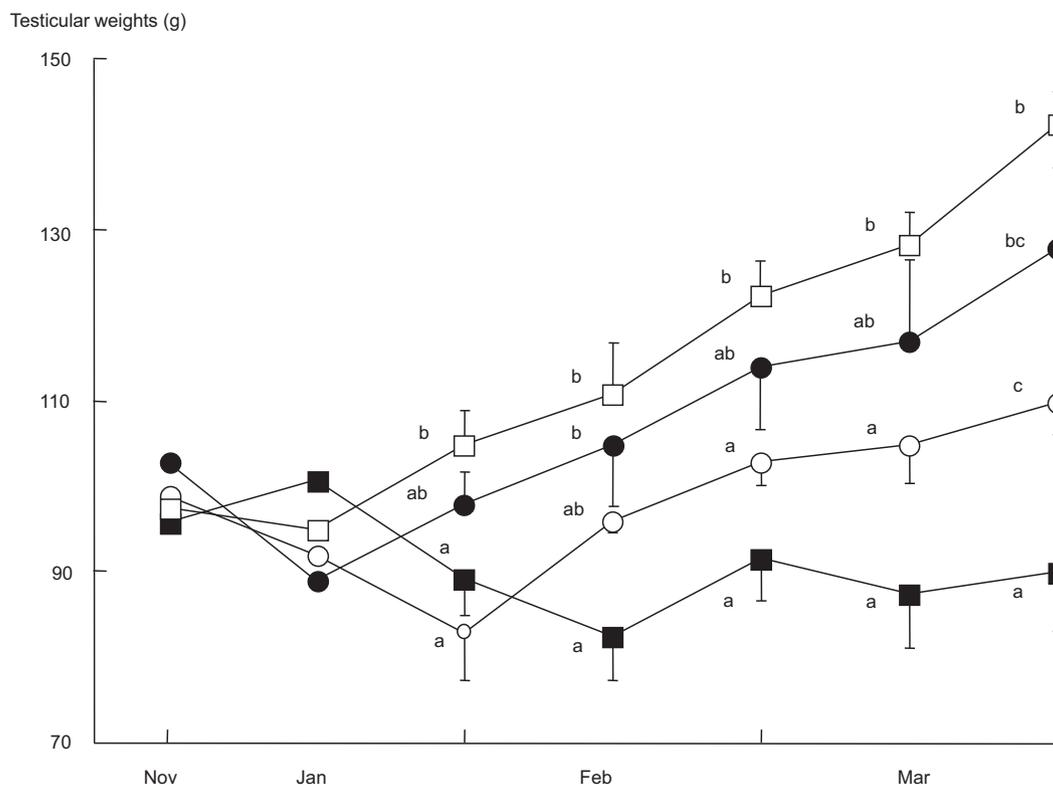
differed among groups ( $P < 0.01$ ). Indeed, pregnancy rates in the females joined by males from the long-day confined and long-day + supplementation groups were greater than in those joined by males from the long-day and control groups ( $P < 0.05$ ; Table 2). Pregnancy rates were also greater in females joined by the males from the long-day group than in those joined by males from the control group ( $P < 0.01$ ).

#### Experiment 2

**Pregnancy rates:** Pregnancy rates were greater in goats joined by males from the long-day + supplementation group (78%) than in those joined by males from the control group (0%;  $P < 0.001$ ).

#### Discussion

The results of these studies confirmed our hypothesis that nutritional supplementation improved the sexual activity of bucks submitted to artificially long days in a semi-extensive management system and their ability to stimulate reproduction in out-of-season goats in semi-extensive or confined management systems. Indeed, in experiment 1, the proportion of pregnant goats was greater in females joined by males from the long-day confined and long-day + supplementation groups than in those joined by males from the long-day and control groups. Interestingly, in experiment 2, in which males from the long-day + supplementation group remained with females in the semi-extensive management system during and after the photoperiod treatment, the proportion of pregnant goats was greater than in those joined by control males. To our knowledge, these are the first studies showing that nutritional supplementation improves the sexual activity



**Fig. 4.** Testicular weights (mean  $\pm$  SEM) in four groups of male goats. Three groups were kept in semi-extensive management and grazed daily from 1000 h to 1700 h. One group did not receive any treatment (control group; ■). Two other groups were subjected to artificially long days (16 h of light by day) between 15 November and 15 January, followed by natural variations in the photoperiod. After the end of long days, one group did not receive any nutritional supplementation (long-day group; ○), whereas bucks from the other group were individually supplemented with 600 g of a commercial concentrate (long-day + supplementation group; ●). The fourth group was kept in confined conditions, exposed to the same artificially long days as the other two groups and fed 2 kg of alfalfa hay per animal daily (long-day confined group; □). Testicular weights were determined every two weeks. Different letters indicate significant differences among groups ( $P < 0.05$ ).

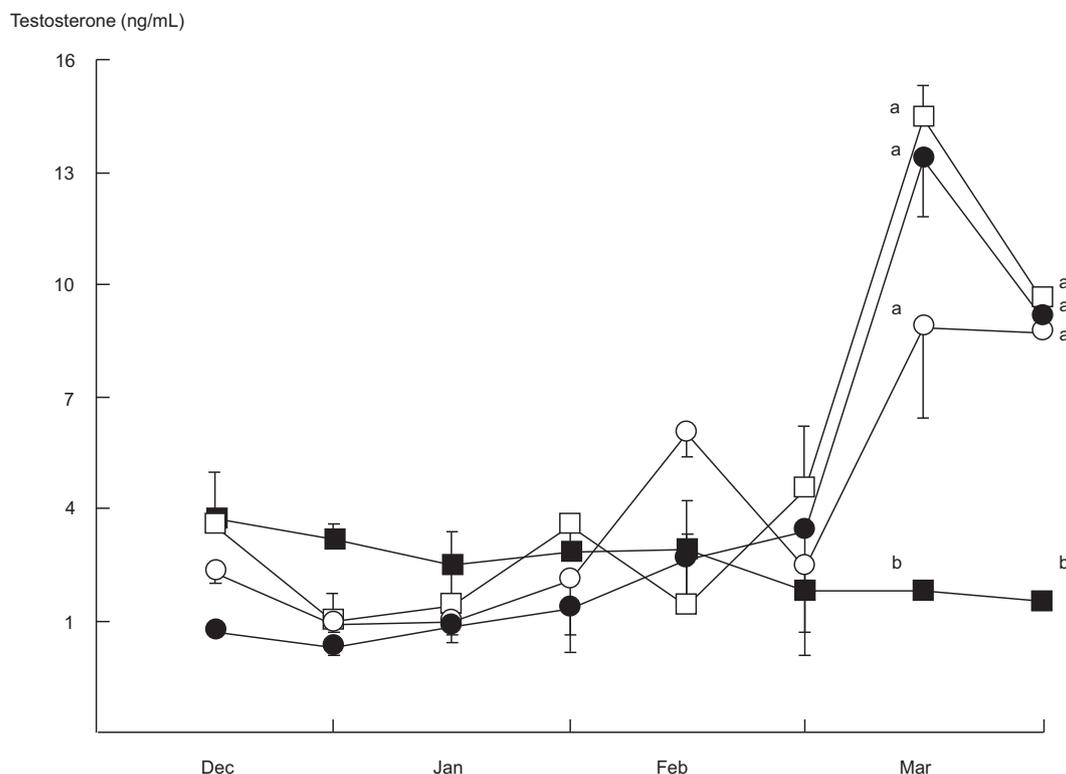
of males submitted to long days in a semi-extensive management system and then their ability to stimulate reproduction of out-of-season goats in semi-extensive or confined management systems.

In experiment 1, BC was greater in the long-day confined group than in the other three groups. In contrast, BW did not differ in most individual values among the three photoperiod-treated groups, despite the different nutritional conditions. In males from these groups, BW increased in January after the end of the long-day treatment, whereas in the control males, this increase occurred one month later, as reported previously (Walkden-Brown et al., 1994; Delgado et al., 2002). In well-nourished male goats from subtropical latitudes kept under natural photoperiod variations, BW starts to increase after the winter solstice, i.e., during the period of increasing day length; in contrast, in those submitted to artificially long days in autumn and winter, BW increases after the end of long days (Walkden-Brown et al., 1994; Delgado et al., 2002). In both situations, it was likely that natural increasing days or artificially long days increased food intake and/or food efficiency with a subsequent increase in BW (Walkden-Brown et al., 1994; Delgado et al., 2002).

In the three photoperiod-treated groups, testicular weights started to increase in February, reaching maximum values in March, as reported previously (Delgado et al., 2002). In contrast, in the control group, this increase did not occur. The increase in testicular weight observed in the three photoperiod-treated groups occurred after the increase in BW, aligning with the results of previous studies (Delgado et al., 2002 and 2020). It appears that in male goats from subtropical latitudes, testicular weight increases after the increase in BW, independent of LH secretion (Walkden-Brown et al., 1994; Hötzel et al., 1995). This could explain why in our study, plasma concentrations of testosterone were lower and did not differ among the four groups from December to

February, despite the increase in testicular weight registered in the photoperiod-treated males. In these males, plasma concentrations of testosterone increased dramatically in mid-March, and there were no differences among groups, but these concentrations were higher than those of the control group, as already reported (Delgado et al., 2002). The lack of differences among the photoperiod-treated males was an unexpected result because there is evidence that in bucks, undernutrition decreases plasma concentrations of testosterone (Walkden-Brown et al., 1994). Interestingly, when housed with females, the photoperiod-treated males displayed more nudging events than the control males, probably due to the high plasma testosterone concentrations. However, this sexual behavior was modified by the nutritional conditions, and it was more intense in the long-day + supplementation group than in the long-day group. These results agreed with those indicating that undernutrition dramatically reduces the sexual behavior of male goats (Martin and Walkden-Brown, 1995; Delgado et al., 2020). Taken together, these findings indicate that artificially long days stimulate an increase in testicular weight, plasma concentrations of testosterone and sexual behavior in male goats kept in semi-extensive management systems.

All females joined by the photoperiod-treated males ovulated, whereas none of females did so when joined by control males. This high ovulatory response indicated that the sexual behavior displayed by males from the three photoperiod-treated groups was enough to stimulate ovulations. Indeed, the sexual behavior displayed by males was a crucial factor in stimulating reproductive activity in seasonally anestrus goats (Martínez-Alfaro et al., 2014; Chales et al., 2016; Zarazaga et al., 2019). However, pregnancy rates were higher in females joined by males from long-day confined and long-day + supplementation groups than in those joined by males from long-day or control groups. These



**Fig. 5.** Testosterone plasma concentrations (mean  $\pm$  SEM) in four groups of male goats. Three groups were kept in semi-extensive management and grazed daily from 1000 h to 1700 h. One group did not receive any treatment (control group; ■). Two other groups were subjected to artificially long days (16 h of light by day) between 15 November and 15 January, followed by natural variations in the photoperiod. After the end of long days, one group did not receive any nutritional supplementation (long-day group; ○), whereas bucks from the other group were individually supplemented with 600 g of a commercial concentrate (long-day + supplementation group; ●). The fourth group was kept in confined conditions, exposed to the same artificially long days as the other two groups and fed 2 kg of alfalfa hay per animal daily (long-day confined group; □). Plasma concentrations of testosterone were determined every two weeks. Different letters indicate significant differences among groups ( $P < 0.05$ ).

**Table 1**

Total number of nudging displayed by long-day treated or control male goats in the presence of females. Males were exposed to artificially long days (16 h of light per day) from 15 November to 15 January.

Groups of males	n	Total number of nudging
Control	3	0 <sup>a</sup>
Long-day	3	114 <sup>b</sup>
Long-day + supplementation	3	540 <sup>c</sup>
Long-day confined	3	752 <sup>d</sup>

<sup>a-d</sup>Values within a column with different superscripts differ significantly at  $P < 0.01$ .

**Table 2**

Ovulatory response and pregnancy rates of seasonal anestrus goats joined by long-day treated or control male goats. Males were exposed to artificially long days (16 h of light per day) from 15 November to 15 January.

Goats joined by males	n	Goats with ovulations (%)	Pregnancy rates (%)
Control	27	0/27 (0) <sup>a</sup>	0/27 (0) <sup>a</sup>
Long-day	27	27/27 (100) <sup>b</sup>	10/27 (37) <sup>b</sup>
Long-day + supplementation	27	27/27 (100) <sup>b</sup>	19/27 (70) <sup>c</sup>
Long-day confined	27	27/27 (100) <sup>b</sup>	24/27 (89) <sup>d</sup>

<sup>a-d</sup>Values within a column with different superscripts differ significantly at  $P < 0.05$ .

differences cannot be explained by different ovulatory responses to the introduction of the photoperiod-treated males because most females that were housed with treated males ovulated. Rather, these findings strongly indicated that nutritional supplementation improved

spermatogenic activity in males submitted to the long-day treatment, consequently improving their ability to fertilize females. This hypothesis is supported by the fact that in rams receiving nutritional supplementation for 7–9 weeks, testicular volume was significantly increased and quantitative and qualitative sperm production improved, probably by decreasing apoptosis in germ cells (Martin and Walkden-Brown, 1995; Guan et al., 2015). Hence, our findings indicated that in our experimental conditions, the combination of photoperiod and nutritional supplementation was a good strategy for increasing the capacity of male goats maintained in semi-extensive management system to fertilize females kept in confined conditions.

In experiment 2, the proportion of pregnant goats was greater in females joined by males from the long-day + supplementation group than in those joined by males from the control group. These results confirmed those obtained in experiment 1. Interestingly, in experiment 2, males remained continuously present with females during and after the photoperiod treatment and were able to stimulate the reproductive activity of seasonally anestrus goats, whereas the continuous presence of control males did not have the same effect. These findings agreed with those indicating that in goats, the previous separation between sexes is unnecessary to stimulate the sexual activity of females when sexually active males are used (Delgado et al., 2015; Zarazaga et al., 2017). The main outcome of this study is, therefore, the demonstration for the first time that the sexual activity of males and females can be stimulated in animals maintained in semi-extensive management systems. The advantage of this demonstration is that the manipulation of reproduction in goat flocks can be achieved without major modifications to the production system. In addition, this technique combining photoperiod, nutritional supplementation, and sociosexual interactions

will allow small producers to transform the reproductive cycle of their flocks with minimal economic investments.

## Conclusion

We conclude that nutritional supplementation improves the ability of bucks submitted to long days in semi-extensive management to stimulate reproduction of seasonally anestrus goats kept in semi-extensive or confined management systems.

## Ethics approval

The experimental procedures used in the present experiment were in accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals (Secretaría de Agricultura et al., 2001).

## Data and model availability statement

None of the data were deposited in an official repository.

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## Author contributions

José Alberto Delgadillo: Conceptualization, methodology, resources, writing – original draft preparation, writing – review & editing. Pablo Iván Sifuentes: Conceptualization, investigation, writing – review & editing. Manuel de Jesús Flores: Investigation, writing – review & editing. Leocici Areli Espinoza-Flores: Conceptualization, investigation, writing – review & editing. Jenifer Denisse Andrade-Esparza: Conceptualization, investigation, writing – review & editing. Horacio Hernández: Conceptualization, formal analysis, writing – review & editing. Matthieu Keller: Conceptualization, methodology, writing – review & editing. Philippe Chemineau: Conceptualization, methodology, writing – review & editing.

## Declaration of interest

Authors declare that there are not conflict of interest.

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## References

Andrade-Esparza, J.D., Espinoza-Flores, L.A., Hernández, H., Chemineau, P., Keller, M., Delgadillo, J.A., 2018. Extensive management conditions do not modify the frequency

- of short ovulatory cycles in progesterone-treated does exposed to sexually active males. *Animal Reproduction Science* 199, 40–44. <https://doi.org/10.1016/j.anireprosci.2018.10.004>.
- Araya, J., Bedos, M., Duarte, G., Hernández, H., Keller, M., Chemineau, P., Delgadillo, J.A., 2016. Maintaining bucks over 35 days after a male effect improves pregnancy rate in goats. *Animal Production Science* 57, 2066–2071. <https://doi.org/10.1017/AN16194>.
- Bedos, M., Duarte, G., Flores, J.A., Fitz-Rodríguez, G., Hernández, H., Vielma, J., Fernández, I.G., Chemineau, P., Keller, M., Delgadillo, J.A., 2014. Two or 24 h of daily contact with sexually active males results in different profiles of LH secretion that both lead to ovulation in anestrus goats. *Domestic Animal Endocrinology* 48, 93–99. <https://doi.org/10.1016/j.domaniend.2014.02.003>.
- Bedos, M., Muñoz, A.L., Orihuela, A., Delgadillo, J.A., 2016. The sexual behavior of male goats exposed to long days is as intense as during their breeding season. *Applied Animal Behavior Science* 184, 35–40. <https://doi.org/10.1016/j.applanim.2016.08.002>.
- Chasles, M., Chesneau, D., Moussu, C., Delgadillo, J.A., Chemineau, P., Keller, M., 2016. Sexually active bucks are efficient to stimulate female ovulatory activity during the anestrus season also under temperate latitudes. *Animal Reproduction Science* 168, 86–91. <https://doi.org/10.1016/j.anireprosci.2016.02.030>.
- Chemineau, P., Pellicer-Rubio, M.T., Lassoued, N., Khaldi, G., Monniaux, D., 2006. Male-induced short oestrous and ovarian cycles in sheep and goats: a working hypothesis. *Reproduction, Nutrition, Development* 46, 417–429.
- Delgadillo, J.A., Martin, G.B., 2015. Alternative methods for control of reproduction in small ruminants: A focus on the needs of grazing industries. *Animal Frontiers* 5, 57–65. <https://doi.org/10.2527/af.2015-0009>.
- Delgadillo, J.A., Canedo, G.A., Chemineau, P., Guillaume, D., Malpoux, B., 1999. Evidence for an annual reproductive rhythm independent of food availability in male creole goats in subtropical northern Mexico. *Theriogenology* 52, 727–737.
- Delgadillo, J.A., Flores, J.A., Véliz, F.G., Hernández, H., Duarte, G., Vielma, J., Poindrón, P., Chemineau, P., Malpoux, B., 2002. Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days. *Journal of Animal Science* 80, 2780–2786.
- Delgadillo, J.A., Cortez, M.E., Duarte, G., Chemineau, P., Malpoux, B., 2004. Evidence that the photoperiod controls the annual changes in testosterone secretion, testicular and body weight in subtropical male goats. *Reproduction, Nutrition, Development* 44, 183–193.
- Delgadillo, J.A., Flores, J.A., Hernández, H., Poindrón, P., Keller, M., Fitz-Rodríguez, G., Duarte, G., Vielma, J., Fernández, I.G., Chemineau, P., 2015. Sexually active males prevent the display of seasonal anestrus in female goats. *Hormones and Behavior* 69, 8–15. <https://doi.org/10.1016/j.yhbeh.2014.12.001>.
- Delgadillo, J.A., Lemièrre, A., Flores, J.A., Bedos, B., Hernández, H., Vielma, J., Guerrero-Cervantes, M., Zarazaga, L.A., Keller, M., Chemineau, P., 2020. Undernutrition reduces the body weight and testicular size of bucks exposed to long days but not their ability to stimulate reproduction of seasonally anestrus goats. *Animal* <https://doi.org/10.1017/S1751731120001329>.
- Duarte, G., Flores, J.A., Malpoux, B., Delgadillo, J.A., 2008. Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. *Domestic Animal Endocrinology* 35, 362–370. <https://doi.org/10.1016/j.domaniend.2008.07.005>.
- Duarte, G., Nava, M.P., Malpoux, B., Delgadillo, J.A., 2010. Ovulatory activity of female goats adapted to the subtropics is responsive to photoperiod. *Animal Reproduction Science* 120, 65–70. <https://doi.org/10.1016/j.anireprosci.2010.04.004>.
- Fitz-Rodríguez, G., De Santiago-Miramontes, M.A., Scaramuzzi, R.J., Malpoux, B., Delgadillo, J.A., 2009. Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect. *Animal Reproduction Science* 116, 85–94. <https://doi.org/10.1016/j.anireprosci.2009.01.004>.
- Garnier, D.H., Cotta, Y., Terqui, M., 1978. Androgen radioimmunoassay in the ram: results of direct plasma testosterone and dehydroepiandrosterone measurement and physiological evaluation. *Annales de Biologie Animale, Biochimie, Biophysique* 18, 265–281.
- González de Bulnes, A., Santiago-Moreno, J., López-Sebastián, A., 1998. Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. *Small Ruminant Research* 27, 243–250.
- Guan, Y., Liang, G., Hawken, P.A.R., Malecki, I.A., Cozens, G., Vercoe, P.E., Martin, G.B., Guan, L.L., 2015. Roles of small RNAs in the effects of nutrition on apoptosis and spermatogenesis in the adult testis. *Scientific Reports* 5, 10372. <https://doi.org/10.1038/srep10372>.
- Hochereau-De Reviers, M.T., Copin, M., Seck, M., Monet-Kuntz, C., Cornu, C., Fontaine, I., Perreau, C., Eisen, J.M., Boomarov, 1990. Stimulation of testosterone production by PMSC injection in the ovine male: effect of breed and age and application to males carrying or not carrying the “F” Booroola gene. *Animal Reproduction Science* 23, 21–32. [https://doi.org/10.1016/0378-4320\(90\)90012-5](https://doi.org/10.1016/0378-4320(90)90012-5).
- Hötzel, M.J., Walkden-Brown, S.W., Blackberry, M.A., Martin, G.B., 1995. The effect of nutrition on testicular growth in mature Merino rams involves mechanisms that are independent of changes in GnRH pulse frequency. *Journal of Endocrinology* 147, 75–85. <https://doi.org/10.1016/j.yhbeh.2018.10.004>.
- Martin, G.B., Walkden-Brown, S.W., 1995. Nutritional influences on reproduction in mature male sheep and goats. *Journal of Reproduction and Fertility* 49, 437–449.
- Martínez-Alfaro, J.C., Hernández, H., Flores, J.A., Duarte, G., Fitz-Rodríguez, G., Fernández, I.G., Bedos, M., Chemineau, P., Keller, M., Delgadillo, J.A., Vielma, J., 2014. Importance of intense male sexual behavior for inducing the preovulatory LH surge and ovulation in seasonally anovulatory female goats. *Theriogenology* 82, 1028–1035.
- National Research Council (NRC), 2007. *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids*. National Academies Press, Washington, DC, USA.

- Oldham, C.M., Adams, N.R., Gherardi, P.B., Lindsay, D.R., McKintosh, J.B., 1978. The influence of level of feed intake on sperm-producing capacity of testicular tissue in the ram. *Australian Journal of Agricultural Research* 29, 173–179.
- Pellicer-Rubio, M.T., Leboeuf, B., Bernelas, D., Forgerit, Y., Pougard, J.L., Bonné, J.L., Senty, E., Chemineau, P., 2007. Highly synchronous and fertile reproductive activity induced by the male effect during deep anoestrus in lactating goats subjected to treatment with artificially long days followed by natural photoperiod. *Animal Reproduction Science* 98, 241–258.
- Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio, diario oficial de la federación. Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, México, Mexico.
- Shelton, M., 1960. Influence of the presence of a male goat on the initiation of oestrous cycling and ovulation of Angora does. *Journal of Animal Science* 19, 368–375.
- Simões, J., Almeida, J.C., Baril, G., Azevedo, J., Fontes, P., Mascarenhas, R., 2007. Assessment of luteal function by ultrasonographic appearance and measurement of corpora lutea in goats. *Animal Reproduction Science* 97, 36–46.
- System Statistics, 2009. Cranes Software International Ltd., San Jose, CA, USA.
- Walkden-Brown, S.W., Restall, B.J., Henniawati, 1993. The male effect in the Australian cashmere goat. 3. Enhancement with buck nutrition and use of oestrous females. *Animal Reproduction Science* 32, 69–84.
- Walkden-Brown, S.W., Restall, B.J., Norton, B.W., Scaramuzzi, R.J., Martin, G.B., 1994. Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. *Journal of Reproduction and Fertility* 102, 351–360.
- Walkden-Brown, S.W., Restall, B.J., Scaramuzzi, R.J., Martin, G.B., Blackberry, M.A., 1997. Seasonality in male Australian cashmere goats: Long term effects of castration and testosterone or oestradiol treatment on changes in LH, FSH and prolactin concentrations, and body growth. *Small Ruminant Research* 26, 239–252.
- Zarazaga, L.A., Gatica, M.C., Hernández, H., Gallego-Calvo, L., Delgado, J.A., Guzmán, J.L., 2017. The isolation of females from males to promote a later male effect is unnecessary if the bucks used are sexually active. *Theriogenology* 95, 42–47. <https://doi.org/10.1016/j.theriogenology.2017.02.023>.
- Zarazaga, L.A., Gatica, M.C., Hernández, H., Chemineau, P., Delgado, J.A., Guzmán, J.L., 2019. Photoperiod-treated bucks are equal to melatonin-treated bucks for inducing reproductive behaviour and physiological functions via the “male effect” in Mediterranean goats. *Animal Reproduction Science* 202, 58–64. <https://doi.org/10.1016/j.anireprosci.2019.01.008>.