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Undernutrition reduces the body weight and testicular size of bucks exposed to long days but not their ability to stimulate reproduction of seasonally anestrous goats

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*In semiarid conditions, feed is often scarce and variable with underfeeding being common; these factors can potentially induce fertility reductions in both sexes. Sexually active bucks are able to very efficiently fertilize out-of-season goats, but we do not know whether underfeeding would reduce the ability of bucks to fertilize goats during these periods. Two experiments were conducted to determine (i) testicular size and change of odor intensity of undernourished bucks exposed to long days and (ii) the ability of these bucks to stimulate reproductive activity in seasonally anestrous goats. In experiment 1, bucks (*n = 7*) were fed 1.5 times the normal maintenance requirements from September to May and formed the well-fed group. Another group of bucks (*n = 7*) were fed 0.5 times the maintenance requirements and formed the undernourished group. All bucks were subjected to artificially long days from 1 November to 15 January; this period was followed by a natural photoperiod until 30 May. Body weight, scrotal circumference and male odor intensity changes were determined every 2 weeks. In experiment 2, two groups of female goats (*n = 26 each*) were exposed to well-fed (*n = 2*) or undernourished bucks (*n = 2*) on 31 March. Ovulations and pregnancy rates were determined by transrectal ultrasonography. In experiment 1, a treatment by time interaction was detected for BW, scrotal circumference and odor intensity changes (*$P < 0.001$*). The BWs of well-fed bucks were greater than those of the undernourished bucks from October to May (*$P < 0.01$*), as were the scrotal circumferences from December to March (*$P < 0.05$*) and odor intensities from February to May (*$P < 0.05$*). In experiment 2, the proportions of females that ovulated at least once (100%* v. *96%) or those that were diagnosed as pregnant (85%* v. *77%;* $P > 0.05$*) did not differ significantly between the goats exposed to well-fed or undernourished bucks. The interval between the introduction of bucks and the onset of estrous behavior was shorter in goats exposed to well-fed bucks compared to the interval for those goats exposed to undernourished bucks (2.5 ± 0.2* v. *9.5 ± 0.6 days;* $P < 0.05$*). We conclude that undernourishment reduces the testicular size and odor intensity responses in bucks exposed to long days, but that undernourished bucks are still able to stimulate reproductive activity in seasonally anestrous goats, as is also the case for well-fed bucks.*

Keywords: caprine, photoperiod, male effect, nutrition, pregnancy

Implications

These results show that undernourishment reduces testicular size and odor intensity in bucks exposed to long days in the autumn-winter period to stimulate their sexual activity during the sexual rest interval. Nonetheless, these undernourished bucks are able to stimulate reproductive activity in goats during the seasonal anestrous period as effectively as well-fed bucks exposed to the same photoperiodic treatment. These results strongly indicate that this photoperiodic treatment could be used for bucks kept under extensive management conditions to stimulate reproductive activity in females during seasonal anestrous.

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Introduction

Reproductive seasonality is a common characteristic of most goat breeds adapted to subtropical latitudes (Restall, 1992; Walkden-Brown *et al.*, 1994; Duarte *et al.*, 2008). In Criollo goats from subtropical northern parts of Mexico, for example, the annual photoperiodic cycle is the main environmental cue that controls the timing of the breeding season in both males and females (Delgadillo *et al.*, 2004; Duarte *et al.*, 2010). However, this timing can be modified by social interactions between males and females. Indeed, in seasonally anestrous goats, sexual activity is stimulated within the first 5 days after joining with males (Shelton, 1960; Chemineau *et al.*, 2006; Bedos *et al.*, 2014). This phenomenon is called the male effect (Delgadillo *et al.*, 2009). The sexual response of female goats exposed to males can be modified by factors such as the sexual behaviors displayed by bucks. Thus, well-fed bucks exposed to long days in the autumn-winter period exhibit increased plasma testosterone concentrations and display intense sexual behavior in the spring (Delgadillo *et al.*, 2002; Chasles *et al.*, 2016; Zarazaga *et al.*, 2017). Photostimulated, sexually active bucks are able to induce more seasonally anestrous goats to ovulate than the photoperiodically untreated sexually inactive goats (>90% *v.* <10%; Delgadillo *et al.*, 2002; Muñoz *et al.*, 2016).

The nutritional status of bucks kept under natural photoperiods dramatically modifies the duration and timing of the breeding season as well as their ability to stimulate the ovulatory activity of seasonally anestrous goats. Indeed, in Australian cashmere bucks, undernourishment delays the increase in and advances the decline of testicular sizes, plasma testosterone concentrations and odor intensity during the breeding season. In addition, the amplitudes of the fluctuations of these sexual variables are lower in well-nourished bucks (Walkden-Brown *et al.*, 1994). Moreover, these undernourished bucks induce a lower proportion of goats to ovulate and result in lower pregnancy rates than the well-nourished bucks when females are exposed to the male effect (Walkden-Brown *et al.*, 1993a). The reduced ability of undernourished bucks to stimulate the sexual and reproductive activities of female goats could be due to their low plasma testosterone concentrations, weak sexual behaviors and odor intensities (Walkden-Brown *et al.*, 1993a and 1994). Nevertheless, we do not know whether testicular size and odor intensity are altered in undernourished bucks exposed to long days and whether these bucks are able to stimulate sexual activity in anestrous goats as effectively as the well-nourished bucks are able to do by compensating for nutritional deficiencies by responses to photoperiodic treatment. Thus, we hypothesize here that (i) the testicular sizes and odor intensity responses of undernourished bucks to photoperiodic treatments would be lower than those of the well-nourished bucks and (ii) these bucks would be less able to stimulate reproductive activity of anestrous goats compared to well-fed bucks. To test these possibilities, we performed two experiments. In experiment 1, we determined the scrotal circumference and odor intensity changes of both undernourished and well-fed bucks exposed to long days in the autumn-winter period. In experiment 2, we determined the ability of the undernourished bucks exposed to long days to stimulate reproductive activity in anestrous female goats in comparison with well-fed bucks.

Material and methods

General conditions of study

The study was carried out in the Laguna region of the State of Coahuila, Mexico (Latitude, 26°23′ N; Longitude, 104°47′ W). At this latitude, the photoperiod varies from 1336 h on the summer solstice to 1024 h on the winter solstice; all animals maintained in open barns followed these variations in day length. We used local animals from the same region, which were previously described as Criollo goats (Duarte *et al.*, 2008).

Experiment 1

Nutritional and photoperiodic treatments. We used two groups of sexually experienced male goats ($n = 7$ each), which were 3 years old at the beginning of study. In this population, the rest season in males lasts from January to June (Delgadillo *et al.*, 1999). Bucks of each group were housed in separate shaded open pens (6 × 6 m each) and were balanced for BW, body condition (**BC**) and scrotal circumference. The well-fed group received a high level of nutrition consisting of alfalfa hay (2.3 Mcal/kg, 18% CP per kg of DM) and 200 g of commercial concentrate (1.7 Mcal/kg, 14% CP per kg of DM) which provided 1.5 times the maintenance requirement (NRC, 2007), BW: 51 ± 0.6 kg, BC: 2.7 ± 0.1 and scrotal circumference: 27 ± 0.5 cm (mean ± SEM). The undernourished group received a low level of nutrition consisting of oat hay (1.8 Mcal/kg, 7% CP per kg of DM), which provided 0.5 times the maintenance requirement (NRC, 2007), BW: 50 ± 1.6 kg, BC: 2.7 ± 0.1 and scrotal circumference: 27 ± 0.4 cm. The animals were fed as a group and feeding was adjusted every 2 weeks in relation to the BW to maintain a corresponding level of nutrition. All bucks had free access to mineral blocks and water. The bucks were maintained in open barns and were exposed to long days in autumn and winter to stimulate their sexual activity as described by Delgadillo *et al.* (2002). The bucks were subjected to artificial long days (e.g. 16 h of light/8 h of darkness) from 1 November to 15 January. Artificial light was supplied from 0600 to 0800 h and from 1800 to 2200 h to extend the natural day. The light on and off periods were regulated by an electronic timer; the light intensity was at least 300 lx laterally to the eyes of the animals. After 15 January, the males were returned to natural photoperiodic variations until the end of the study on 30 May. In well-fed bucks, this photoperiodic treatment generally takes approximately 6 weeks to increase plasma testosterone concentrations and improves the sexual behavior of males, as demonstrated by our research group (Delgadillo *et al.*, 2002; Ponce *et al.*, 2014).

Measurements

Body weights. Body weights were determined before the distribution of diets using an electronic balance with a capacity of 250 kg and a precision of 0.05 kg (Torrey, Nuevo León, México).

Body conditions. Body conditions were determined using a scale from 1 (very lean) to 4, based on lumbar palpation performed by an experienced technician (Walkden-Brown *et al.*, 1997); this technician performed these assessments during the entire experiment.

Testicular sizes. Testicular sizes were measured as scrotal circumferences using a flexible tape graduated in centimeters. Light pressure was used on the tape to pull the testes together and the equatorial region of both testicles was measured (Oldham *et al.*, 1978). Scrotal circumference is related to testicular weight, which is a good indicator of spermatogenetic activity size and weight (Delgadillo *et al.*, 1999; Oldham *et al.*, 1978).

Male odor intensity. Male odor intensity changes were determined using a score of 0 (neutral odor, no different from a female or a castrated male), 1 (mild male odor intensity), 2 (moderate male odor intensity) and 3 (strong male odor intensity). Odor intensity was evaluated by smelling the dorsum of the neck 10 to 15 cm behind the base of the horns (Walkden-Brown *et al.*, 1994). The same person determined all of these variables every 2 weeks from September (scrotal circumference) or November (odor intensity) to May.

Experiment 2

Conditions of female goats. We used multiparous, seasonally anovulatory female goats that were 3 to 4 years old at the beginning of study. For this population, the anestrous season in females was described previously and lasts from February to August (Duarte *et al.*, 2008). Females were offered daily 2 kg of alfalfa hay (2.3 Mcal/kg, 18% CP per kg of DM) and 200 g of commercial concentrate (1.7 Mcal/kg, 14% CP per kg of DM) per animal for maintaining adult weight (NRC, 2007) and had free access to mineral blocks and water. These animals were fed as a group. These females had delivered between October and December and were hand-milked once a day during the study. From December until 30 March, all females were isolated from the males. On 10, 20 and 30 March, the ovulatory status of goats was assessed by transrectal ultrasonography using an Aloka SSD-500 device (Aloka Co., Ltd, Tokyo, Japan) connected to a transrectal 7.5-MHz linear probe (Simões *et al.*, 2007). Females with no corpora lutea in the three observations were considered to be anovulatory. All females were, therefore, diagnosed as anovulatory. On 30 March, these females were separated into two groups ($n = 26$ each) and were balanced for BW and BC (BW: 40 ± 1; BC: 2.7 ± 0.1, both groups); the females remained in open shaded pens (20×20 m) under natural photoperiodic conditions. The distance between groups was greater than 100 m, thus preventing the risk of interference between treatments (Walkden-Brown *et al.*, 1993b).

The male effect

The induction of out-of-season ovulatory activity by using the male effect is a well-known technique described in the literature and is widely used in the laboratory. It consists of putting males and females in direct contact when the females are anovulatory, so that the sudden introduction of males very rapidly (<48 h) induces ovulations in the majority of females. On 31 March (day 0), one group of goats ($n = 26$) was exposed to the photostimulated, well-fed bucks ($n = 2$), whereas the other group ($n = 26$) was exposed to the photostimulated, undernourished bucks ($n = 2$). Bucks were selected at random from the well-fed and undernourished groups used in experiment 1. The males remained in contact with females for 18 consecutive days and were present among the females in the same group. Each group of goats was divided into two subgroups by a wooden barrier to prevent fighting between males. Hence, each buck was in contact with 13 females and the males were rotated daily within their respective groups to avoid individual effects. During the male effect, the well-fed and undernourished bucks received the high and low nutrition levels, respectively, described in experiment 1. Considering the expected results in the present study, we estimated that the number of males and females used in each group would allow for significant differences in sexual behavior, ovulatory response to the male effect and the proportions of pregnant goats, as described in previous studies (Bedos *et al.*, 2010 and 2014).

Measurements

Sexual behavior of bucks. Sexual behavior displayed by bucks in the presence of females was determined from 0830 to 0900 h during the first 3 days after their introduction into the groups of females. Trained observers followed the bucks individually and recorded the number of nudging and anogenital sniffing events (Bedos *et al.*, 2016). The measurement of the sexual behavior of bucks allows evaluating if males are active toward the females with whom they were put in contact (Bedos *et al.*, 2016).

Estrous behavior, ovulations and pregnancy rates. Estrous behavior was registered twice daily by direct visual observations from 0800 to 0900 h and from 1800 to 1900 h during the entire experiment. A female was considered to be in estrus if she stood immobile when mounted by a buck. The durations of estrous cycles were classified as short (<17 days) or normal (17 to 25 days; Chemineau *et al.*, 1992). Ovulations and ovulation rates were determined by transrectal ultrasonography at 5 and 20 days after the males were introduced (Simões *et al.*, 2007). In addition, ovulations were confirmed by the plasma progesterone concentrations in blood samples taken daily from each female from day 1 to day 18 after the bucks were introduced. All 5 ml samples were collected by jugular venipuncture, placed in tubes containing 30 μl of heparin and centrifuged immediately at

3500 **g** for 30 min. The obtained plasma was stored at −20°C until the progesterone concentrations were measured by immunoenzymatic assay (Canépa *et al.*, 2008). The sensitivity was 0.25 ng/ml. The intra- and inter-assay CVs were 7% and 10%, respectively. Females with plasma progesterone concentrations ≥1.0 ng/ml were considered to have ovulated (Chemineau *et al.*, 1992). Pregnancy rates were determined by the presence of embryos as observed by transrectal ultrasonography 50 days after the introduction of males in both groups (González de Bulnes *et al.*, 1998).

Statistics analysis
In experiment 1, the BW, BC, testicular circumference and male odor values were analyzed using two-way repeated measures ANOVA to detect any differences between treatments. The model included treatment (group), sampling time (weeks) and the interactions between these factors. The *t*-test and Mann–Whitney *U* test (males' odor) were used for 2 × 2 individual point comparisons. In experiment 2, the proportions of females displaying estrous behavior or ovulations and pregnancy rates were compared between groups using the χ^2 test. The ovulation rates were compared using the Mann–Whitney *U* test. The male behavioral components were analyzed with a χ^2 test for goodness of fit considering a random distribution of 50% in each group when comparing photostimulated, well-fed and undernourished bucks. All statistical analyses were performed using the statistical package SYSTAT 13 (2009). The results were expressed as the mean ± SEM, and differences were considered significant at a level of $P \leq 0.05$.

Results

Experiment 1
Body weight. A treatment by time interaction was detected for BW ($P < 0.001$), thus necessitating examination of treatment effects over time (Figure 1). In both well-fed and undernourished bucks, BW did not differ in September. Thereafter, in well-fed bucks, BW progressively increased and reached a maximum value in mid-March and then decreased until the end of the study. In contrast, in the undernourished bucks, BW remained at the same level throughout the study. Body weight was greater in the well-nourished bucks than undernourished bucks from mid-October to May ($P < 0.05$).

Body condition. A treatment by time interaction was detected for BC ($P < 0.001$; Figure 2). In the well-fed bucks, BC remained high and exhibited no important changes throughout the study. In contrast, in the undernourished bucks, BC decreased progressively until February and then remained low until May. Therefore, the BC of well-fed bucks was greater than for undernourished bucks from October to May ($P < 0.01$).

Scrotal circumference. A treatment by time interaction was detected for scrotal circumference ($P < 0.001$; Figure 3). In

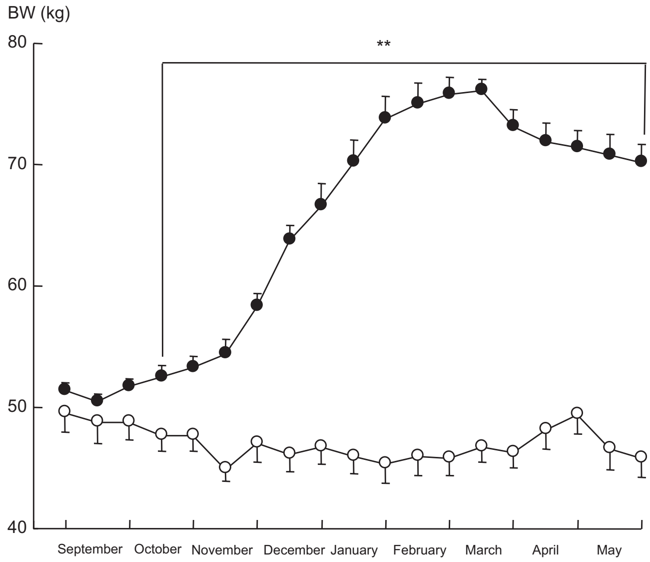

Figure 1 Body weights (mean ± SEM) in two groups of male goats subjected to artificially long days (16 h of light per day) between 1 November and 15 January followed by natural photoperiodic variations. One group of bucks ($n = 7$) was fed 1.5 times the normal maintenance requirements, for example, the well-fed group (•). The other group of bucks ($n = 7$) was fed 0.5 times the maintenance requirements, for example, the undernourished group (○). Body weight was determined every 2 weeks. The stars indicate significant differences between the two groups (** $P < 0.01$).

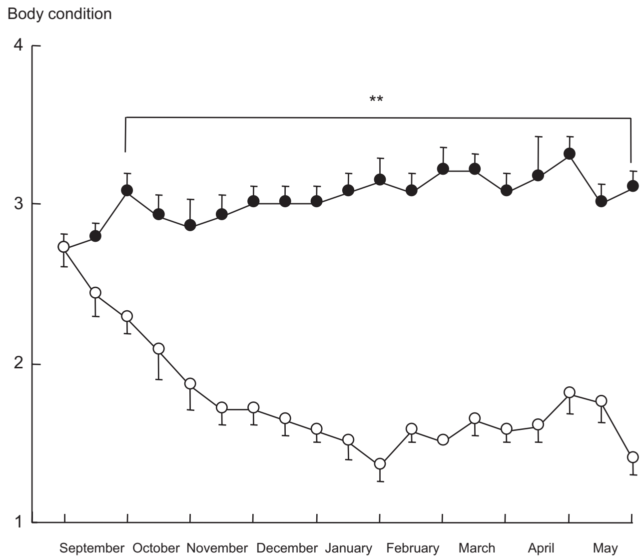

Figure 2 Body conditions (mean ± SEM) in two groups of male goats subjected to artificially long days (16 h of light per day) between 1 November and 15 January followed by natural photoperiodic variations. One group of bucks ($n = 7$) was fed 1.5 times the normal maintenance requirements, for example, the well-fed group (•). The other group ($n = 7$) was fed 0.5 times the maintenance requirements, for example, the undernourished group (○). Body condition was determined every 2 weeks. The stars indicate significant differences between the two groups (** $P < 0.01$).

well-fed bucks, the scrotal circumferences decreased from September to November and then increased to reach a maximum value on March. Afterward, this variable decreased until May. In contrast, in the undernourished bucks, scrotal circumferences decreased from September to January and

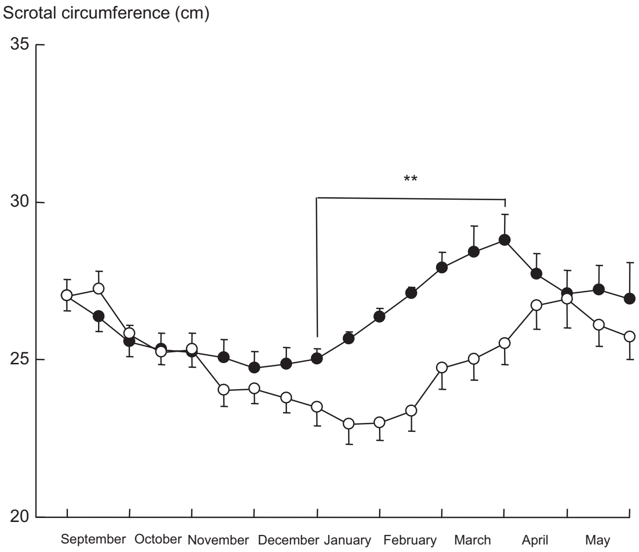

Figure 3 Scrotal circumferences (mean ± SEM) in two groups of male goats subjected to artificially long days (16 h of light per day) between 1 November and 15 January followed by natural photoperiodic variations. One group of bucks ($n = 7$) was fed 1.5 times the maintenance requirements, for example, the well-fed group (•). The other group ($n = 7$) was fed 0.5 times the maintenance requirements, for example, the undernourished group (○). Scrotal circumferences were determined every 2 weeks. The stars indicate significant differences between the two groups (** $P < 0.01$).

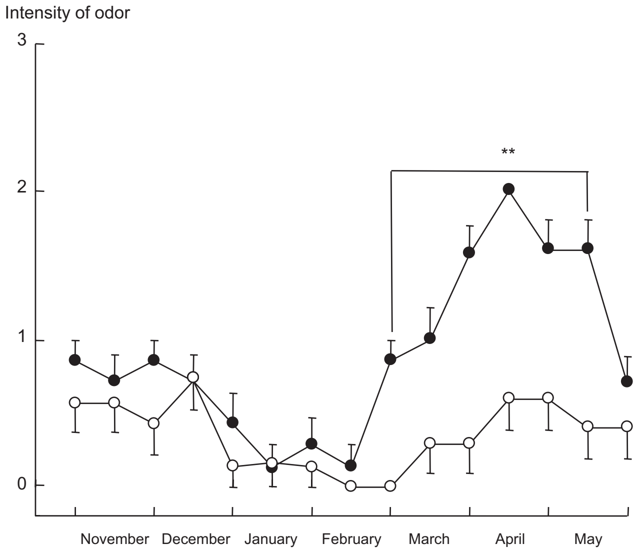

Figure 4 Intensities of male odor (mean ± SEM) in two groups of male goats subjected to artificially long days (16 h of light per day) between 1 November and 15 January followed by natural photoperiodic variations. One group of bucks ($n = 7$) was fed 1.5 times the normal maintenance requirements, for example, the well-fed group (•). The other group ($n = 7$) was fed 0.5 times the maintenance requirements, for example, the undernourished group (○). Male odor determinations were made every 2 weeks. The stars indicate significant differences between the two groups (** $P < 0.01$).

then increased to reach a maximum value on April. Afterward, this variable decreased until May. The scrotal circumferences of well-fed bucks were greater than for the undernourished bucks from January to March ($P < 0.01$).

Odor intensity. A treatment by time interaction was detected for odor intensity ($P < 0.001$; Figure 4). In both well-fed and undernourished bucks, the odor intensity decreased from November to February and did not differ between the two groups ($P > 0.05$). Afterward, the odor intensity increased in both groups of bucks and reached maximum values in April. However, the odor intensity was greater in the well-fed bucks than in the undernourished bucks from March to May ($P < 0.01$).

Experiment 2

Sexual behavior of bucks. The well-fed bucks displayed more nudging and anogenital sniffing events than the undernourished bucks (e.g. 310 and 307 total events *v.* 9 and 136, respectively; $P < 0.05$).

Estrus and ovulatory responses of goats to the male effect. The proportions of females displaying estrous behavior or ovulated throughout the study did not differ between the goats exposed to well-fed or those exposed to undernourished bucks (estrous behavior: 88% in both groups; females that ovulated: 100% *v.* 96%, respectively; $P > 0.05$). However, the distribution of estrus and ovulations after the introduction of males and the association of estrus and ovulations differed between the females in contact with well-fed or undernourished bucks (Figure 5; Table 1). The

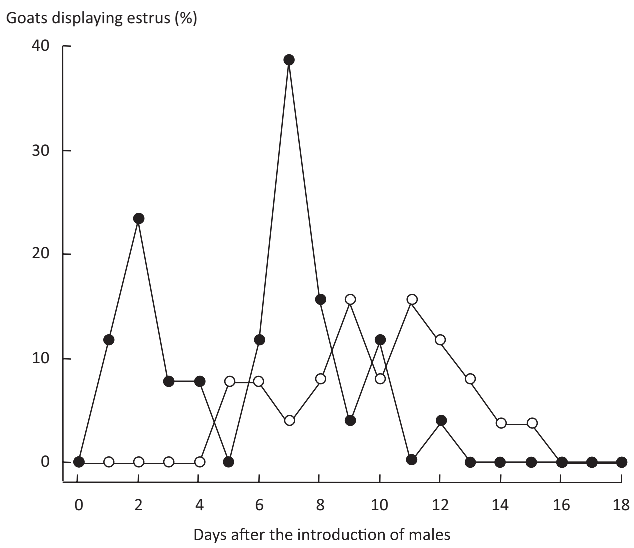

Figure 5 Daily percentages of goats displaying estrous behavior when exposed to male goats previously subjected to artificially long days (16 h of light per day) between 1 November and 15 January followed by natural photoperiodic variations. One group of goats ($n = 26$) was exposed on 31 March to the well-fed bucks ($n = 2$; •), whereas the other group ($n = 26$) was exposed to the undernourished bucks ($n = 2$; ○). Estrous behavior was determined twice per day for 18 days. Day 0 is the day on which the bucks were introduced to the groups of goats.

interval between the introduction of bucks and the onset of estrous behavior was shorter in those goats exposed to well-fed bucks than in those exposed to undernourished bucks (2.5 ± 0.2 *v.* 9.5 ± 0.6 days; $P < 0.05$). In addition, the proportion of goats that displayed short estrous cycles

Table 1 *Estrus and ovulatory responses of female goats exposed to well-fed or undernourished bucks during 18 days*

	Days 0 to 4				Days 5 to 18			
	Goats displaying				Goats displaying			
Goats exposed to	Estrus with ovulations (%)	Estrus without ovulations (%)	Ovulations without estrus (%)	Total responsiveness goats (%)	Estrus with ovulations (%)	Estrus without ovulations (%)	Ovulations without estrus (%)	Total responsiveness goats (%)
Well-fed bucks	11/26[a] (42)	2/26[a] (8)	10/26[a] (38)	23/26[a] (88)	23/26[a] (88)	3/26[a] (12)	0/26[a] (0)	26/26[a] (100)
Undernourished bucks	0/26[b] (0)	0/26[a] (0)	10/26[a] (38)	10/26[b] (38)	23/26[a] (88)	0/26[a] (0)	1/26[a] (4)	24/26[a] (92)

[a,b]Values within a row with different superscripts differ significantly at $P < 0.01$.

was higher in goats exposed to well-fed males than in those exposed to undernourished bucks (46% *v.* 4%; $P < 0.05$). The percentage of estrus associated with ovulations during the first 4 days after introducing the males was higher in goats exposed to well-fed bucks than in goats exposed to undernourished bucks (Table 1, $P < 0.05$). Similarly, the ovulation rate during the first 4 days was higher in goats joined with the well-fed bucks than in those joined with the undernourished bucks (1.8 ± 0.1 *v.* 1.2 ± 0.1, respectively; $P < 0.05$). Finally, the proportion of females diagnosed pregnant did not differ between goats in contact with well-fed or undernourished bucks (85% *v.* 77%, respectively; $P < 0.05$).

Discussion

The results of the present study confirmed our first hypothesis that the response of undernourished bucks to photoperiodic treatment is lower than the response of well-fed bucks. Indeed, the scrotal circumference, odor intensity and sexual behavior of males when in contact with females were lower in the undernourished bucks than in the well-fed bucks. Unexpectedly, the undernourished bucks were able to stimulate the reproductive activity of female goats, as did the well-fed bucks, thus rejecting our second hypothesis. Surprisingly, the proportions of goats displaying estrous behavior or ovulations and those diagnosed pregnant did not differ between females exposed to undernourished or well-nourished bucks. Nonetheless, the estrous response pattern of females strongly differed between those exposed to the well-fed bucks or to the undernourished bucks.

In experiment 1, the data showed that the amplitude and timing of scrotal and odor intensity differed markedly between the well-fed and undernourished bucks. The increase in scrotal circumference and odor intensity was delayed in the undernourished bucks compared with the well-fed bucks. In addition, the maximum values of scrotal circumference and odor intensity were lower in the undernourished bucks than in the well-fed bucks. These differences could be explained by various factors. First, it was demonstrated that in subtropical bucks and rams, testicular size is dramatically influenced by BW (Walkden-Brown *et al.*, 1994; Hötzel *et al.*, 1995). Generally, in well-nourished bucks from subtropical latitudes kept under natural photoperiodic variations, testicular sizes increase from January to May after the increase in BW that is probably induced by increased food intake (Delgadillo *et al.*, 1999). Similarly, in photoperiod-treated bucks, testicular sizes also increased after the increase of BW that was observed during and just after the end of the long days (Delgadillo *et al.*, 2002). In both cases, this first step in testicular growth seemed to be independent of LH secretion as reported in other subtropical bucks and rams (Walkden-Brown *et al.*, 1994; Hötzel *et al.*, 1995). Therefore, in our study, the very low BC registered during the entire study in the undernourished bucks probably prevented increases in scrotal circumference during and after the end of the long days. This hypothesis is supported by findings showing that in Merino rams artificially exposed to a subtropical photoperiod and fed with a Mediterranean diet inducing important variations in BW, the changes of scrotal circumference paralleled changes in BW but not those of photoperiod (Martin *et al.*, 2002). Second, in bucks and rams, undernourishment enhanced the negative testosterone feedback on LH secretion and as consequence, the LH plasma concentrations were lower in the undernourished males than in the well-nourished males (Walkden-Brown *et al.*, 1994; Martin and Walkden-Brown, 1995). In our study, it is likely that undernourishment enhanced the negative feedback of testosterone on LH secretion, reduced the growth of scrotal circumference and then prevented it from reaching the levels observed in well-nourished bucks after the end of the long days. Third, the odor intensity of bucks is testosterone-dependent and increases after the increase of plasma testosterone concentrations during the natural breeding season or approximately 6 weeks after the end of the artificially long days (Walkden-Brown *et al.*, 1994; Rivas-Muñoz *et al.*, 2007). In our study, the odor intensity of the undernourished bucks after the end of long days was lower than that of the well-fed males. This difference could be related to low plasma testosterone concentrations in undernourished bucks in comparison with the well-nourished bucks, as has been reported for other subtropical bucks (Walkden-Brown *et al.*, 1994). Taken together, our data show that artificial long days stimulate the growth of scrotal circumference and odor intensity of both well-nourished and undernourished bucks. Nonetheless, the amplitude was smaller and the timing of the response of undernourished bucks to the photoperiodic treatment was delayed compared to the well-fed bucks.

These findings indicate that even if the photoperiod is the main factor for timing the breeding season in subtropical bucks, nutrition is a strong modulator, as was reported earlier for bucks and rams from subtropical latitudes (Walkden-Brown *et al.*, 1994; Martin *et al.*, 2002).

In experiment 2, our data indicated that undernourished bucks exposed to long days were able to stimulate reproductive activity in anestrous goats, as was also the case for the well-nourished bucks. Nonetheless, the sexual response throughout the study strongly differed between groups of goats. The goats ovulated rapidly after joining the well-fed bucks and displayed short estrous and ovulatory cycles in the first 11 days after the introduction of bucks: the first one days 0 to 4 and the second one days 5 to 11 (Chemineau *et al.*, 2006; Pellicer-Rubio *et al.*, 2007). In contrast, for those goats in contact with undernourished bucks, these two peaks of sexual activity were not observed and estrus and ovulations occurred only from 5 to 15 days after male introduction, as was reported for cashmere goats exposed to undernourished bucks (Walkden-Brown *et al.*, 1993a). In seasonally anestrous goats, male sexual behavior is an important factor for stimulating estrus and ovulatory activities (Delgadillo *et al.*, 2002; Chasles *et al.*, 2016). In our study, the undernourished bucks displayed dramatically decreased sexual behavior and dramatic differences in sexual odor compared to the well-nourished bucks. Therefore, it is likely that the sexual stimulation behavior displayed by the undernourished bucks and their sexual odor were not strong enough to stimulate sexual activity in the same manner that the well-fed bucks could. It is likely that the weak sexual stimulus provided by the undernourished bucks delayed the sexual response and prevented the short estrous and ovulatory cycles, as was previously reported (Walkden-Brown *et al.*, 1993a; Rivas-Muñoz *et al.*, 2007). However, even if only approximately 40% of the female goats in the presence of undernourished bucks ovulated during the first 5 days, we wonder why nearly all of them did ovulate during the following 13 days. This may not be due to the sexual activity of the undernourished bucks themselves but rather to the entraining effect of the 40% of goats which responded during the first wave.

Nevertheless, most goats in contact with either well-fed or undernourished bucks were diagnosed pregnant with the same ratio in both groups and their fertility was similar to that described in other experiments (Fitz-Rodríguez *et al.*, 2009; Bedos *et al.*, 2010). It would be useful to confirm these results in the future by using more than two males per group and by measuring fertility at kidding. Nevertheless, this suggests that undernourished bucks produced spermatozoa of good fertilizing ability from their testes and epididymis when the male effect was active.

Conclusions

Overall, we conclude that in the conditions of our study, undernourishment reduces testicular circumferences and odor intensities of bucks exposed to artificially long days followed by natural photoperiodic changes to stimulate their sexual activity during the sexual rest period. Nonetheless, these undernourished bucks are able to stimulate sexual and reproductive activities in seasonal anestrous goats by means of the buck effect as effectively as the well-fed bucks. Our data strongly suggest that bucks maintained exclusively in extensive conditions, in which their BC is low and similar to that in the present study, should be able to induce sexual activity of seasonally anestrous goats after a photoperiodic treatment with long days in the autumn and winter months. This could cause the preparation of bucks to be more flexible when they remain in extensive management conditions, thus reducing the cost of food for bucks exposed to long days indoors.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Ethics statement

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, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001).

Software and data repository resources

None of the data were deposited in an official repository.

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