

Effects of season and feeding level on reproductive activity and semen quality in Payoya buck goats

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Abstract

The aim of this study was to determine if there is a seasonal pattern of reproductive activity in male Payoya goats and if this seasonality can be modulated by a higher level of nutrition. For a period of 16 months, 10 adult bucks were divided into two experimental groups that differed in their feeding level. The high nutrition group (H, $n = 5$) received 1.6 times their maintenance food requirements. The control nutrition group (C, $n = 5$) received a diet that supported 1.1 times their maintenance requirements. Body weight and testosterone concentrations were determined weekly, and testicular weight was determined every 2 weeks. Sexual behaviour and semen characteristics were determined monthly. Feeding level did not affect the onset or the end of the reproductive activity as measured by testosterone concentrations, with high testosterone concentrations between July and November. Ejaculation latency was positively influenced by feeding level: 43.2 ± 2.2 s vs. 61.6 ± 3.2 s for H and C group, respectively ($P < 0.001$). The percentage of males that ejaculated or that were sexually active was higher in the H group ($P < 0.01$). No differences between feeding levels were observed in the different semen characteristics studied. However, major differences between months were observed for all studied variables. These results lead us to conclude that Payoya bucks exhibit large seasonal variation in their reproductive activity. Higher feeding level allowed a better sexual behaviour in bucks in late spring, when male effect is used on the local livestock to breed females.

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1. Introduction

Productivity in small ruminants is affected by the seasonality of reproduction. In rams and goat bucks, a decrease in quantitative and qualitative semen production and sperm fertility during the non-breeding season has been reported (bucks: [1]; rams: [2]). Photoperiod or annual season has been suggested as the principal factor influencing seasonality of reproduction in bucks at high latitudes [3]. However, other environmental stimuli,

such as availability of food and social interactions [4,5], should not be disregarded as potential regulators of the seasonality of reproduction.

Nutrition is considered to be an important factor affecting seasonality of reproductive functions in bucks [6]. In the Mediterranean area, the majority of goats are maintained in extensive or semi-extensive systems, where food availability is highly dependent on the season. Hence, it is often thought that nutrition may be responsible for seasonal reproductive patterns. However, since changes in photoperiod also occur during times of scarce nutrition, it is possible that season and nutrition have complex effects on reproductive activity. Delgado et al. demonstrated that even at low latitudes

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(26° N), male Creole goats fed constantly showed seasonality in their reproductive activity [7], indicating that photoperiod is the most important factor controlling reproductive activity in male goats [8]. However, they did not study the possible effects of high feeding levels to try to reduce this reproductive seasonality.

Although there is a wealth of information on the reproductive patterns of various goat breeds at middle (>25°) and high (>40°) latitudes [9,10], very little information exists on Spanish goat breeds. This study looked at the Payoya goat, an endangered Spanish goat breed adapted to the environmental conditions of the Mediterranean area.

The objective of this study was to determine whether or not there is a true seasonal pattern of reproductive activity in the male Payoya goat independent of variations in food availability, and if this seasonality can be reduced by a higher level of nutrition.

2. Material and methods

2.1. General

This experiment was performed in accordance with the Spanish Animal Protection Policy RD1201/2005, which conforms to the European Union Directive 86/609 regarding the protection of animals used in scientific experiments.

The study was conducted on an experimental farm of the University of Huelva (latitude 37°15') which meets the requirements of the European Community Commission for Scientific Procedure Establishments (1986). The male Payoya goats ($n = 10$) used in the study were placed under intensive conditions when they were 6–8 months old, and they were at least 1 year old at the beginning of the 21-month experimental period (January 2002–September 2003). During whole experiment they were kept separated from females.

At the start of the experiment, bucks were pubertal but were growing. For this reason, from 4 January, 2002 to 31 May, 2002, when the animals' live weight was at least 80% of the adult live weight [11] (61.1 ± 1.7 kg; 31 May, 2002), the feeding level was adjusted to allow appropriate development of all animals. On 31 May, 2002, bucks were divided into two groups of similar body weight (BW) and testicular weight (TW) [12] (Table 1). Each group received a different level of feeding. The high nutrition group (H, $n = 5$) received a mean daily intake of 1.6 times their maintenance food requirements and the control group (C, $n = 5$) received a mean daily intake of food that was 1.1 times their maintenance requirements. Therefore, the H group was

Table 1

Mean (\pm S.E.M.) for initial and final body weight (BW, kg) and testicular weight (TW, g), ejaculation latency (s), ejaculating males (%) and active males (%) for Spanish Payoya bucks fed at 1.6 \times maintenance (H group) or 1.1 \times maintenance (C, group).

	H group	C group
Initial BW (kg)	62.3 \pm 1.5	60.8 \pm 3.8
Final BW (kg)	71.7 \pm 0.4 ^c	66.1 \pm 0.6 ^d
Initial TW (g)	260.0 \pm 18.7	285.0 \pm 21.8
Final TW (g)	286.3 \pm 3.4	297.3 \pm 3.9
Ejaculation latency (s)	43.2 \pm 2.2 ^c	61.6 \pm 3.2 ^d
Ejaculating males (%)	89.5 \pm 1.5 ^a	80.6 \pm 2.1 ^b
Active males (%)	92.8 \pm 1.3 ^a	82.9 \pm 2.0 ^b

Values in the same row that have different letters are significant. (a and b): ($P < 0.01$) and (c and d): ($P < 0.001$).

fed with 1.6 kg of concentrate and 0.5 kg of barley straw and the C group was fed with 0.95 kg of concentrate and 0.6 kg of barley straw. These amounts correspond to a daily intake of 4.87 Mcal of metabolisable energy and 101.4 g of digestible protein (H group) and 3.19 Mcal of metabolisable energy and 66.4 g of digestible protein (C group), which is equivalent to 1.6 and 1.1 times respectively the maintenance food requirements for the two groups, based on a standard male goat of 75 kg BW [13]. Concentrate feed was offered once a day and distributed individually to each animal; in addition, barley straw was offered to the group as a whole. The concentrate was a commercial mixture of maize (26.3%), bean (20%), oats (14.1%), cotton-seed (13.7%), pea (13.4%), lupin (7.3%), barley (0.2%), wheat (0.2%), sunflower seeds (0.2%), and a commercial concentrate as mineral–vitamin complement (4.6%). Bucks had free access to water and mineral blocks containing oligoelements.

During the experimental period, animals were permanently kept in communal yards with an uncovered area without any supplementary light.

2.2. Measurements

From January 2002 to September 2003, data were collected but the effect of feeding level was studied only from June 2002 to September 2003. Body weight was determined once a week. Testicular weight was assessed every 2 weeks by comparative palpation with an orchidometer [12]. The determinations were carried out by the same operator throughout the study.

Plasma testosterone concentrations were determined from blood samples obtained once a week at 09:00 by jugular venipuncture throughout the experimental period.

During the first 8 days of each month of the study, sexual behaviour and sperm characteristics were analysed for all males. Each of these 8-day periods was divided into two 3-day periods of daily sperm collection separated by 2 days of rest. Semen from each buck was collected at 09:00, using an artificial vagina (40 °C), into a pre-warmed tube at 35 °C, and it was maintained at this temperature until processing. The tubes of freshly collected semen were immediately transferred to a water bath at 35 °C. The data obtained on the first day of each monthly collection were not considered in the analysis to avoid a possible effect of sexual rest on semen quality [1].

On each occasion, sexual behaviour was assessed by presenting bucks with an intact oestrus-induced doe and giving them 5 min to ejaculate. The ejaculation latency (EL) and the percentage of males refusing to ejaculate were recorded. The induction of oestrus in teaser does was performed with a subcutaneous injection of 2 mg of oestradiol cypionate (Sigma–Aldrich Química, S.A., Spain) [7]. Animals were always tested in the same order and by the same handlers. Prior to the start of the experiment (January 2002), all bucks had been trained to mount a teaser doe and ejaculate into an artificial vagina.

2.3. Semen evaluation

The ejaculated semen volume was recorded immediately after collection in a graduated collection vial. Global motility was assessed by transferring a drop of undiluted semen to a warm slide (35 °C), placing a cover slip on it, and observing it under a microscope at 40× immediately after semen collection. The assessment of global motility was made on the basis of an arbitrary scale from 0 to 5 (0 = no motility, 5 = 100% motility) [14]. Forward progressive motility (FPM) was assessed by transferring a drop of semen diluted 1:400 with PBS onto a warm slide, as described previously, and estimating the progressive sperm motility (scale 0–5) observed at 400× [14]. Moreover, three objective measures of sperm swimming velocity were obtained: (a) curvilinear velocity (VCL, $\mu\text{m/s}$), or the velocity of the actual trajectory of the sperm; (b) straight-line velocity (VSL, $\mu\text{m/s}$), which is the velocity calculated using the straight-line distance between the beginning and end of the sperm track; and (c) average path velocity (VAP, $\mu\text{m/s}$), or the velocity over the smooth calculated path. Sperm concentration was measured in a haemocytometer after diluting an aliquot of semen with a 0.05% formaldehyde saline solution (1:400) and observing at 400× magnification. The total number of spermatozoa per ejaculate was calculated by

measuring the volume and sperm concentration. All parameters were recorded using a computer-aided sperm analyser (Sperm Class Analyzer, Microptic, Barcelona, Spain).

2.4. Blood sampling

Blood samples were collected in tubes containing heparin by jugular venipuncture. Plasma was obtained after centrifugation at $3000 \times g$ for 30 min and then stored at $-20\text{ }^{\circ}\text{C}$ until hormone concentrations were measured. A radioimmunoassay was used to measure plasma testosterone in duplicate in plasma samples of 50 μL as described by Garnier et al. [15]. Sensitivity, defined as two standard deviations from the zero control, was 0.1 ng/mL. The intra-assay coefficient of variation was 8.3%.

2.5. Definitions of reproductive activity

Reproductive state was assessed using characteristics of the testosterone profile. For each buck a period of high endocrine activity was determined. This was assumed to begin on the date of the first plasma testosterone sample above 5 ng/mL [3] in a sequence of two or more samples above 5 ng/mL, and to end on the date of the last plasma testosterone sample above 5 ng/mL in a sequence of two or more samples below 5 ng/mL. We consider this period the main reproductive season.

2.6. Statistical analyses

Because bucks were still growing at the beginning of the study, the effect of feeding level on all studied variables was only analysed only from June 2002 to September 2003. The effects of feeding level and time of year on BW, TW, and plasma testosterone were analysed using analysis of variance (ANOVA) for repeated measures. Increases or decreases in BW, TW, ejaculation latency, ejaculate volume, global motility, forward progressive motility, sperm concentration, total number of spermatozoa per ejaculate, VCL, VSL, and VAP were analysed using ANOVA based on the monthly mean of each parameter. Explanatory variables were feeding level (control group and high group), month (16 months), and the interaction nutrition by month. When ANOVA resulted in significant differences between months, multiple comparisons between means were tested by Tukey's test. The monthly percentage of bucks that ejaculated and the monthly percentage of sexually active males, i.e. bucks that tried to ejaculate at least twice but did not succeed within

5 min, were compared using a χ^2 test. The effect of feeding level and year of experiment on the onset of reproductive activity based on the testosterone concentrations was analysed by ANOVA. Analysis of data was computed using the SPSS package [16].

3. Results

3.1. Effects of nutrition and season on body weight and testicular weight

Fig. 1 shows the mean (\pm S.E.M.) BW (Fig. 1A) and TW (Fig. 1B) throughout the study period. The ANOVA

for repeated measurements showed an effect of the time of the year on BW ($P < 0.001$). Differences between nutritional groups showed a clear effect of feeding level on mean BW from June 2002 to the end of the experiment, with higher BW in the H than in the C group ($P < 0.001$) (Table 1). The highest BW was observed during May 2003 (mid-spring) and the lowest values when the two experimental groups were formed in July 2002 (mid-summer). From June 2002 to September 2003, no effect of nutrition on TW was observed (Table 1), but a clear effect of time on TW was found ($P < 0.001$). The highest TW was observed in September 2003 (late summer, onset of autumn) and the

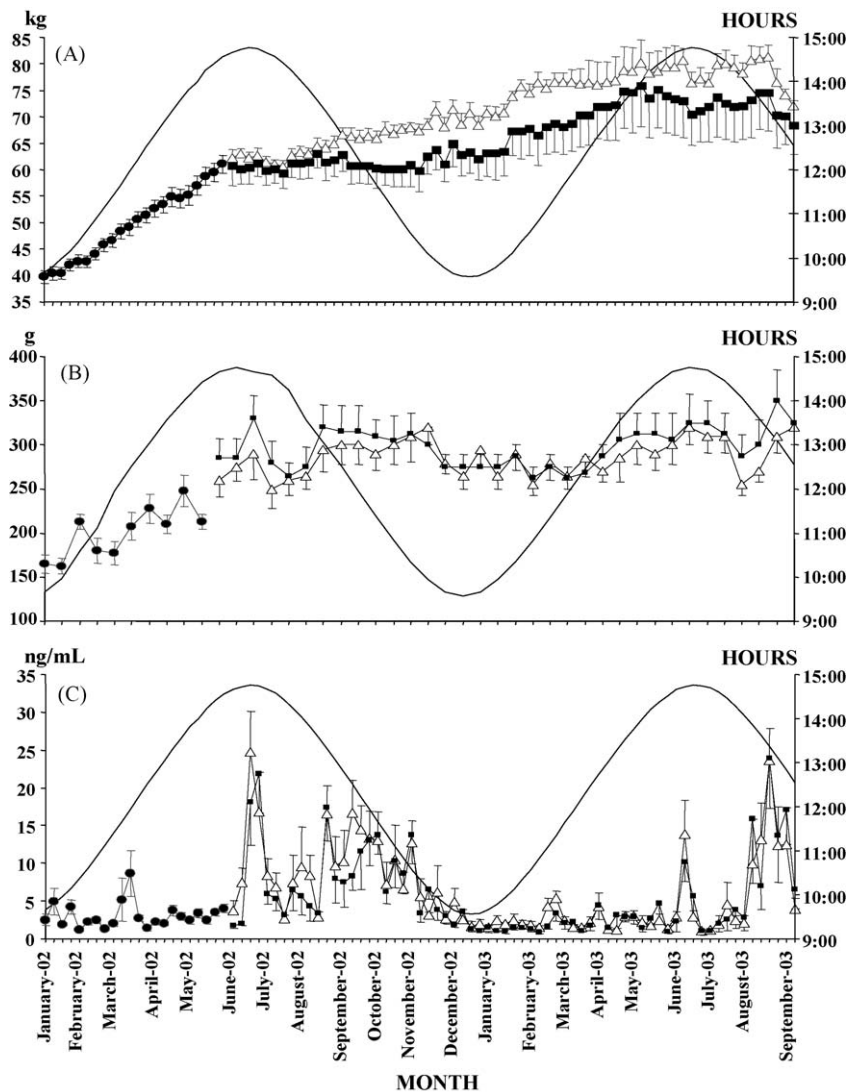


Fig. 1. Mean (\pm S.E.M.) for body weight (kg) (A), testicular weight (g) (B), and testosterone concentrations (ng/mL) (C) for Spanish Payoya bucks fed at $1.6\times$ maintenance (H, Δ) or $1.1\times$ maintenance (C, \blacksquare). The (\bullet) represents the period before the bucks were divided into two groups. The (—) area represents the annual photoperiod curve.

lowest between February and March 2003 (late winter). Interaction effects between treatment and time were not observed on BW or TW.

Significant differences between months were observed in BW gains and losses ($P < 0.001$) despite the constant amount of food provided for each group. Body weight increased during January 2003 (winter season) (105.0 ± 16.8 g/day), remained stable during spring and autumn, and decreased during August 2003 (-164.3 ± 36.8 g/day). Similarly, TW gains/losses varied between months ($P < 0.01$). Testicular weight increased during August 2002 and August 2003 and during March–April 2003, decreasing in November 2002 (-1.1 ± 0.5 g/day).

3.2. Effect of nutrition and season on testosterone concentrations

The weekly mean testosterone concentrations (\pm S.E.M.) for each group are shown in Fig. 1C. No differences between nutritional groups or interactions between nutrition and time were observed, but there was a significant effect of time ($P < 0.001$). From 21 June to 1 November, 2002, and from 8 August, 2003 until the end of experiment, the plasma testosterone concentrations were higher than 5 ng/mL, indicating that the values were highest during summer and autumn months. The values were lowest during spring and winter months ($P < 0.001$).

Neither nutrition level nor the year affected the onset of the reproductive activity according to the testosterone concentrations (2002: 11 July \pm 18.9 days vs. 10 August \pm 10.9 days for H and C groups; 2003: 9 August \pm 3.4 vs. 8 August \pm 8.1 days for the H and C groups). Similarly, the end of sexual activity was not affected by feeding level: 10 November \pm 12.8 days vs. 3 November \pm 11.2 days in the H and C groups, respectively.

3.3. Effect of nutrition and season on mating behaviour

There was a clear effect of nutrition (Table 1) and month on ejaculation latency ($P < 0.001$). The lowest reaction time was observed in November 2002 and the highest in June 2002. Thus, the autumn months were the time when bucks responded fastest to stimulation by an oestrous female, and spring and summer months were the time of year with higher ejaculation latency (Fig. 2).

The percentage of males by month that ejaculated and the percentage of males considered sexually active are shown in Fig. 3. Differences between treatments were observed: a higher percentage of males from the H group ejaculated or were sexually active ($P < 0.01$) (Table 1). For both variables, major differences were observed over the months ($P < 0.001$). The percentage of males that ejaculated was 100% from November 2002 to February 2003 and the percentage of sexually active bucks was maximum from November 2002 to

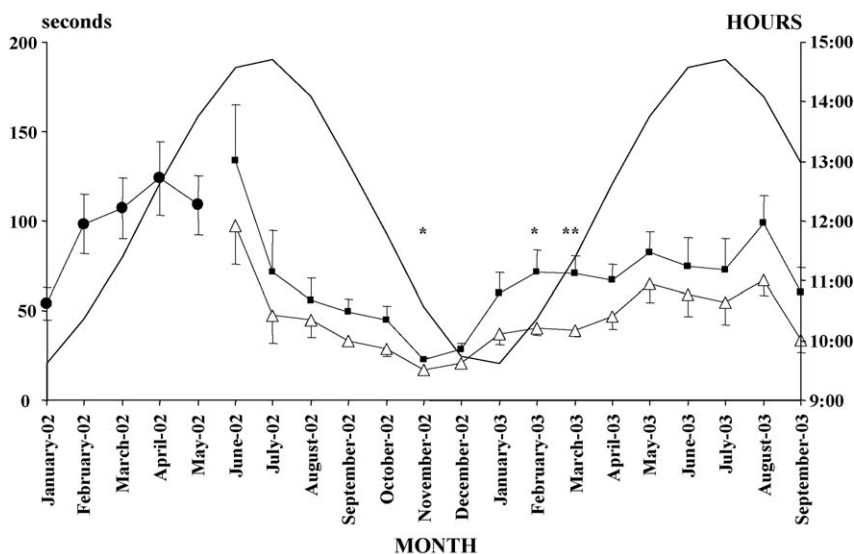


Fig. 2. Monthly mean (\pm S.E.M.) for ejaculation latency (s) for Spanish Payoya bucks fed at 1.6 \times maintenance (H, Δ) or 1.1 \times maintenance (C, \blacksquare). The (\bullet) represents the period before the bucks were divided into two groups. The (—) area represents the annual photoperiod curve. * $P < 0.05$; ** $P < 0.01$.

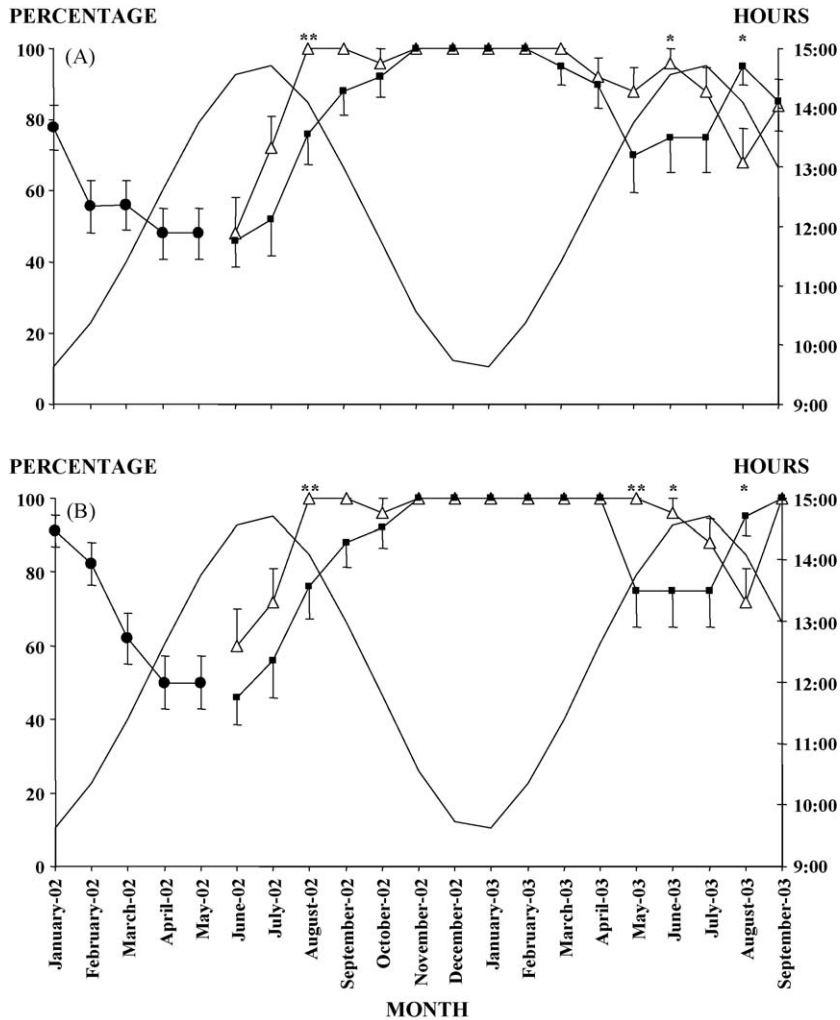


Fig. 3. Monthly mean (\pm S.E.M.) for percentage of Spanish Payoya bucks that ejaculated (A) and males that were sexually active (B) in groups fed at $1.6\times$ maintenance (H, Δ) or $1.1\times$ maintenance (C, \bullet). The (\bullet) represents the period before the bucks were divided into two groups. The (—) area represents the annual photoperiod curve. * $P < 0.05$; ** $P < 0.01$.

April 2003 and in September 2003. The lowest percentages of males that ejaculated or were sexually active were in June 2002. Moreover, an interaction was observed between nutrition and month ($P < 0.01$) (Fig. 3).

3.4. Effect of nutrition and season on semen production and sperm quality

Ejaculate volume, sperm concentration, and total number of spermatozoa per ejaculate did not change significantly between nutritional groups. Interaction of nutrition with month was found for ejaculate volume and total number of spermatozoa per ejaculate ($P < 0.01$) (Fig. 4). For all variables, there was an effect of month ($P < 0.001$). The lowest values for

volume, concentration, and total number of sperm per ejaculate were in June 2002, September 2002, and September 2003, respectively; the highest values were in December 2002, May 2003, and December 2002, respectively.

Global motility and forward progressive motility were higher in the C group than in the H group: 4.57 ± 0.04 and 3.45 ± 0.06 , respectively, in the H group, compared with 4.81 ± 0.03 and 3.85 ± 0.06 in the C group ($P < 0.001$). There was a clear effect of month on forward progressive motility ($P < 0.001$), with higher values in March–April 2003 and lower values in June, August, and September 2002.

No effect of nutrition on motility parameters was observed. However, there was a significant effect of month on VCL, VSL, and VAP ($P < 0.01$). The highest

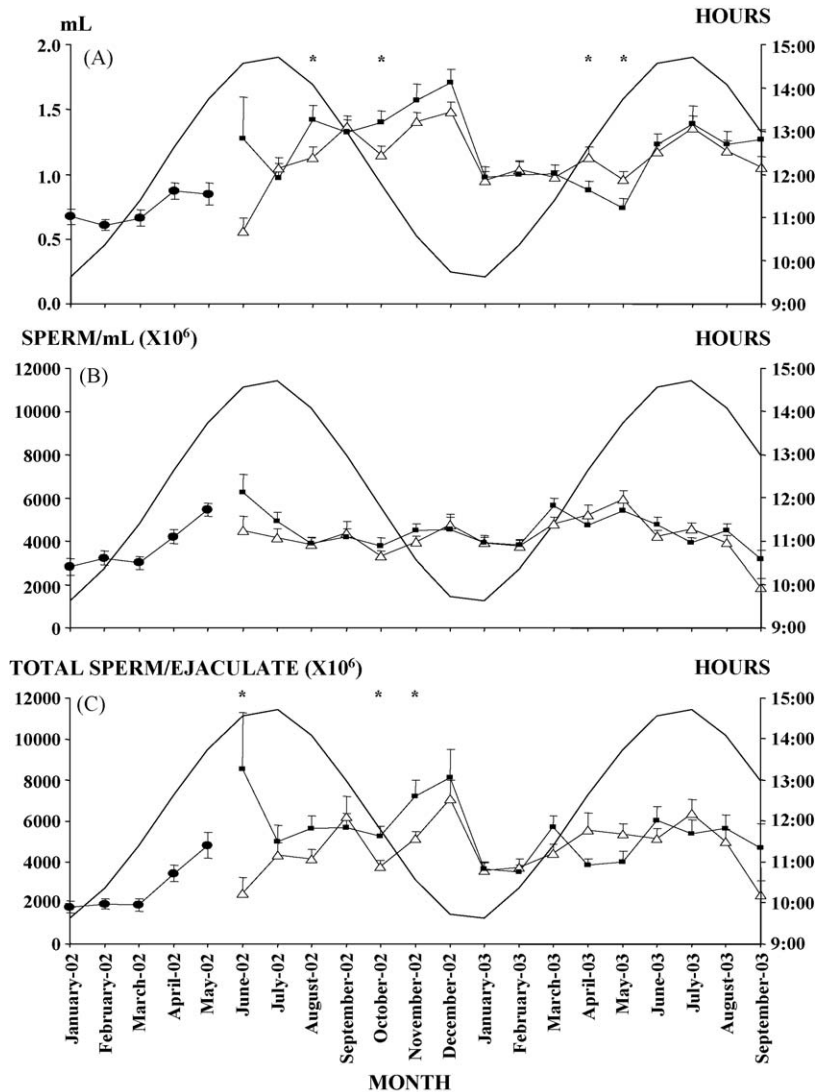


Fig. 4. Monthly mean (\pm S.E.M.) for ejaculate volume (mL) (A), sperm concentration (sperm/mL, $\times 10^6$) (B), and total number of sperm/ejaculate (sperm/ejaculate, $\times 10^6$) (C) for Spanish Payoya bucks fed at 1.6 \times maintenance (H, Δ) or 1.1 \times maintenance (C, \blacksquare). The (\bullet) represents the period before the bucks were divided into two groups. The (—) area represents the annual photoperiod curve. * $P < 0.05$.

values for these variables were observed in March 2003 ($145.2 \pm 3.0 \mu\text{m/s}$, $106.5 \pm 3.4 \mu\text{m/s}$, and $127.1 \pm 3.1 \mu\text{m/s}$ for VCL, VSL, and VAP, respectively). The lowest values were observed in December 2002 ($114.4 \pm 2.8 \mu\text{m/s}$, $85.7 \pm 2.8 \mu\text{m/s}$, and $97.3 \pm 2.9 \mu\text{m/s}$ for VCL, VSL, and VAP, respectively).

4. Discussion

The present study demonstrated that Spanish Payoya bucks maintained under a natural photoperiod show marked seasonal variation in their reproductive performance at latitudes where other breeds are considered to be short seasonal breeders. These changes were

observed despite an increase in the level of nutrition. During the study, seasonal variations in body weight, testicular weight, testosterone secretion, sexual behaviour, and the characteristics of semen were observed. Improved nutrition was observed to affect only ejaculation latency and the percentage of males that ejaculated or were sexually active. Since these changes were observed despite the different and constant levels of feeding given to the animals, the results indicate that season strongly influences the reproductive physiology of these animals, independently of the feeding level.

More food (H) resulted in higher live weight, as expected. Animals from the beginning of the experiment until June 2002 were clearly growing because they

were young at the beginning. For this reason, feeding was adjusted to allow appropriate development of the animals. From June 2002 to the end of the experiment, a constant level of feeding was used for both H and C groups. In all cases, independently of the level of feeding, both groups showed a seasonal pattern in body weight changes. Such changes in BW have previously been reported in this species, not only in males but also in females [6–8,17,18]. The changes were coincident with seasonal reproductive activity: gain in BW was observed when animals were reproductively inactive and BW losses were observed during the breeding season [6,8,18,19]. It has been suggested that differences in food intake might be the reason for the BW changes [19,20]. Accordingly, Barenton et al. demonstrated that photoperiod has a direct effect on body weight: long days stimulate weight gain, whereas short days inhibit it [21].

There was no interaction of nutrition with time of the year on the BW or on the mean change in BW over the experiment, indicating that BW of both groups responded similarly to the two levels of feeding.

Similarly, testicular weight, a good predictor of sperm production at the testis level [22], exhibited significant changes during the study, with a 27% difference between the minimum and maximum values. The seasonal changes were clear throughout the experiment, with decreasing values between September and March (late summer to late winter), and increasing values between April and August (spring to mid-summer), according to Walkden-Brown et al. [6] and Delgado et al. [7]. However, no effect of feeding level on testicular weight was observed in the present study, in contrast to the results reported by Walkden-Brown et al. [6]. In that experiment, the differences in nutritional levels between groups were higher. However, our objective was to determine whether an increase in nutrition that allows normal development could improve the reproductive activity of bucks.

Significant changes were also found in testosterone secretion, which reached maximum levels in summer, but the changes in testosterone secretion were delayed with respect to changes in testicular weight. These results demonstrated the existence of a well-defined breeding season characterised by high testicular weight and high testosterone production. Seasonal changes of testosterone secretion found in the present study were very similar to those reported for local bucks in Mexico [7,8] with a similar method, and recently in other Mediterranean goat breeds [10]. However, in Alpine bucks at 46° N, plasma testosterone increases are delayed until late August–September [23]. Among the

suggested reasons for these differences between the onset of the breeding season in Creole bucks in subtropical Mexico or in our experiment and in Alpine and Saanen bucks at higher latitudes was a shorter lag time between the perception of the photoperiodic signal and the expression of the physiological responses [8].

Ejaculation latency, an index of sexual behaviour, shows clear annual variation. The seasonal changes observed in this study appear to be intermediate between those reported in subtropical areas and those reported for temperate caprine breeds. Indeed, Delgado et al. observed a peak of sexual activity in subtropical areas in July–August [7], whilst at temperate latitudes, intense sexual activity is observed from October to April [1,17]. The feeding level had a clear effect on ejaculation latency, indicating that when the level of nutrition increases, sexual activity also increases. Moreover, this result agrees with the percentage of bucks that ejaculated or were sexually active. These results reinforce previous studies showing that nutrition can be a modulator of the seasonal reproductive cycle in male goats.

It should be noted that in Spain a common practice in the local livestock area is to induce a male effect from February to June depending on each flock. The results of the present study have important practical consequences on such routines, because they indicate that males are in poor reproductive condition during this period. However, if the animals do have good nutritional levels, they will be more sexually active and in better condition for mating.

There was no effect of feeding level on ejaculate volume, sperm concentration, and number of spermatozoa per ejaculate. Our objective with the control group was to characterise reproduction in the Payoya buck, and observe whether seasonality could be modulated with higher amounts of feed. We were not interested in inducing malnutrition in these animals in order to reduce reproductive parameters. The annual variation in these parameters is consistent with other results in the literature. Karagiannidis et al. [24], working with three breeds of goats, reported similar results to those of the present trial with the Alpine goat breed, and the only difference between the present results and those obtained with the Saanen and Damascus breeds is that the latter produced a higher total number of spermatozoa during summer. Similar results have also been reported by Pérez and Mateos [25] and Roca et al. [26] for other Spanish goat breeds.

Several studies have shown a correlation between sperm motility and fertility [27]. Sperm motion parameters are very important for oocyte penetration,

and progressive sperm motility is essential for efficient penetration [28]. Sperm motion parameters were recorded in the present study using CASA, which is more objective than visual assessment. To our knowledge, this is the first report in which computer-assisted analysis was used to study sperm quality of male goats throughout the year. The sperm motion parameters, VCL, VSL, and VAP did not differ significantly between feeding groups according to results obtained for volume, sperm concentration, and number of spermatozoa per ejaculate. The reason could be because the bucks were fed at least at maintenance requirements, allowing all the males to have an adequate seminal quality and in consequence similar sperm motion parameters.

In conclusion, the results of this work support the hypothesis that native Payoya bucks exhibit considerable reproductive seasonality, with an intense sexual activity between August (mid summer) and November (mid-late autumn). The data also suggest that a higher feeding level does not have an evident effect on this seasonality, but that if the animals are in good nutritional condition outside the main reproductive season, they would be more sexually active for mating in late spring, when the male effect is usually applied in the field on the local livestock.

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