Research in Veterinary Science 139 (2021) 177–185

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

The use of photoperiod-treated bucks to induce a "male effect" does not compensate for the negative effects of nutritional restriction of the females in Mediterranean goats

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ARTICLE INFO

Keywords:
Male effect
Seasonality
Photoperiod
Nutrition
NEFA
IGF-1

ABSTRACT

This work examined the effect of acute nutritional restriction or supplementation one week before male introduction on the reproductive performances of the "male effect" when using photostimulated or control males in goats. On 22 March, 84 anoestrous does were placed with photostimulated bucks or with bucks which had received no treatments. One week before male introduction, the females were provided with different nutritional regimes: Supplemented, restricted or control females. The non-esterified fatty acids (NEFAs) and Insulin Growth Factor-1 (IGF-1) concentrations were measured in the same samples. Fecundity, fertility, prolificacy and productivity were also determined. No interaction between both sources of variation was observed in any of the reproductive variables studied. Treatment of the bucks increased the percentage of females expressing behavioural oestrous associated with ovulation (71% *vs* 90% for Natural and Photo groups, respectively, $P < 0.05$). The Supplemented females showed higher ovulation rate than Restricted females (1.77 ± 0.13 *vs* 1.05 ± 0.05, $P < 0.001$), fecundity (71% *vs* 43%, $P < 0.05$); fertility (76% *vs* 29%, $P < 0.05$) and productivity (1.00 ± 0.15 *vs* 0.29 ± 0.11 kids per female, $P < 0.01$). In the Supplemented females, the higher reproductive results could be due to the lower NEFAs and higher IGF-1 concentrations at ovulation and at the time of oestrus compared to the Restricted females. Thus, the present experiment results demonstrate that nutrition is an important factor in the response to the "male effect" at Mediterranean latitudes, and its negative effect cannot be counterbalanced by using photostimulated bucks.

1. Introduction

The seasonality of reproduction is a common feature in breeds of goats from temperate latitudes; the photoperiod is the main environmental factor controlling the hypothalamic-pituitary-gonadal axis (Fatet et al., 2011; Zarazaga et al., 2011a, 2011b, 2011c). However, other environmental stimuli as social interactions or nutrition could be important modulators of this seasonality (Walkden-Brown et al., 1993; Mani et al., 1996).

The introduction of bucks to anovulatory females previously separated from bucks can induce oestrous activity within a few days; this is termed the "male effect". Even reproductive response has been obtained without isolation between bucks and does when sexually active males are used (Gallego-Calvo et al., 2014a). The practice of placing bucks in the presence of does helps optimise the sexual response of does during the anoestrous period, leading to a greater synchronisation of oestrous onset (Delgadillo et al., 2009). It is widely used in extensive and semi-extensive goat production systems in Mediterranean countries. The intensity of the "male effect" depends greatly on factors such as breed, stage of anoestrus, postpartum stage, parity number, nutrition, body condition and the sexual behaviour of the males (Walkden-Brown et al., 1993; Flores et al., 2000; Gallego-Calvo et al., 2015a).

In the last two decades, a considerable number of experiments have demonstrated that the "male effect" using males rendered sexually active by artificial exposure to long days induces more sexual activity in seasonally anoestrous does than when bucks kept under the natural photoperiod are used (Zarazaga et al., 2010, 2018, 2019a, 2019b).

One important factor that can influence the ovulation and pregnancy

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https://doi.org/10.1016/j.rvsc.2021.07.027
Received 24 March 2021; Received in revised form 9 July 2021; Accepted 22 July 2021
Available online 24 July 2021

rates of does exposed to bucks is the nutritional status of the does. Generally, the number of does that displayed oestrous behaviour or ovulated and their ovulation and pregnancy rates when submitted to the "male effect" is reduced in long-term undernourished does (Gallego-Calvo et al., 2015a, 2018; Zarazaga et al., 2017). However, the ovulation rate can be increased by feed supplementation. Indeed, in undernourished females, feed supplementation for 7 or 14 days before the "male effect" stimulus was initiated has resulted in an increased ovulation rate but did not improve the proportion of females with ovulations or showing oestrous behaviour compared with non-supplemented females (Nottle et al., 1997). In goats managed under grazing conditions (with a fluctuant level of nutrition), supplementing diets 7 days before the "male effect" using only photostimulated bucks produced an increased ovulation rate at the first male-induced ovulation, but the stimulatory effect did not persist into the second male-induced ovulation, and no differences were observed between supplemented and non-supplemented females in the whole experiment (De Santiago-Miramontes et al., 2008). Moreover, undernutrition is the main factor associated with embryo loss up to 30 days of pregnancy, probably because of changes in the uterine environment (Mani et al., 1992; Abecia et al., 2006; Martin and Kadokawa, 2006). In undernourished females, feed supplementation has been shown to improve pregnancy rates (Kleemann and Cutten, 1978; Rhind et al., 1989).

Glucose, insulin, non-esterified fatty acids (NEFAs) and Insulin Growth Factor-1 (IGF-1) are the principal metabolites and metabolic hormones involved in the energy levels of ruminants. The NEFAs increase when there is a demand for energy that determines a mobilisation of the adipose tissue (Bowden, 1971). IGF-I has an important role in the control of follicle development and is likely to be a mediator of the effects of dietary intake on the ovulation rate (Muñoz-Gutierrez et al., 2002). In previous experiments (Gallego-Calvo et al., 2015a, 2018), we observed that glucose and insulin showed, as expected, higher concentrations in animals receiving supplements. Recently we have observed no variation of the NEFAs and IGF-1 concentrations when the bucks were introduced with the females at different moments of the oestrous cycle (Zarazaga et al., 2021). However, NEFAs and IGF-1 plasma concentrations showed different behaviour (increases or decreases) related to the "male effect".

The relation between factors, the "male effect" and nutrition, could be the kisspepetin. The ram effect resulted in an increase in KISS1 mRNA in the rostral and mid-arcuate nucleus (De Bond et al., 2013). Even though there are not very much research studying the role of kisspeptin relaying the influence of nutritional status on reproduction, (Backholer et al., 2010) observed that lean sheep had lower KISS1mRNA levels in both the preoptic area and arcuate nucleus compared with control-fed ewes.

To our knowledge there are no studies that simultaneously compare the reproductive results of the "male effect" using photostimulated bucks *vs* bucks showing natural springtime sexual activity on does submitted to acute undernutrition or supplementation for 7 days before male introduction and maintained over time. Our hypothesis was if the photoperiodic stimulation of the sexual activity of the bucks could compensate the negative effects of a low nutritional status of the does on their response to a buck effect or *vice-versa*. The present experiment aimed to determine: 1) how the reproductive performance response to the "male effect" differs depending on the kind of buck used (photostimulated bucks or not) and the level of nutrition received by the does from one week before the onset of the "male effect" 2) if the negative effect of each main factor (restricted nutrition of lower sexual activity of the bucks) could be counterbalanced by the other one. Metabolic and nutritional parameters (NEFAs and IGF-1) were analysed to interpret better the reproductive responses observed.

2. Material and methods

2.1. Study conditions

All animals were housed in pens with an uncovered area and a covered area. All procedures were performed by trained personnel in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013) and in agreement with European Union Directive 86/609. The procedures of the present experiment were evaluated by the qualified organisation of the ethical committee for animal experimentation (CEEA-OH) from the University of Granada and authorised with reference number 297-CEEA-OH-2018 and authorised by the Andalusia Regional Government with the reference number 22/05/2019/094. The study was conducted at the experimental farm of the University of Huelva (37° 20′N, 6° 54′ W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

2.2. Treatment of bucks to induce the "male effect"

Two groups ($n = 8$ each) of sexually experienced Blanca Andaluza bucks were used to induce the "male effect". The bucks of Blanca Andaluza showed an extended period of reproductive seasonality with basal testosterone concentrations from December to July (Gallego-Calvo et al., 2015b). All bucks were 3–5 years old at the beginning of the study. On 19 November, a group of males assigned at random (photoperiod-treated bucks; Photo) housed in open barns were exposed to long photoperiods (16 h light, 8 h dark; lights on at 0600, lights off at 2200) for 84 days. The photoperiod was regulated by an electric timer that controlled white fluorescent strip lights providing approximately 200 lx at the level of the animals' eyes. At the end of the photoperiod treatment (*i.e.*, on 11 February of the following year), the bucks were maintained under natural photoperiod conditions. The other group of bucks was exposed to the natural photoperiod throughout the experiment (untreated bucks; Natural). Bucks of each group were housed in separate pens.

2.3. Nutrition treatments of the does

On 15 March, one week before "male effect" induction, 84 adult (2–6 years old) non-pregnant Blanca Andaluza does in anoestrus were divided into three groups and given different amounts of nutrition for a total of 41 days, until 25 April when the experiment finished (during the first 7 days, the does were kept isolated from the bucks). The dietary treatment regimens that were imposed on does were consistent with Institut National de la Recherche Agronomique (INRA) standards (Morand-Fehr and Sauvant, 1988).

Groups were balanced according to their body weight (BW) and body condition (BC). The BC was scored by lumbar palpation (always performed by the same handler) based on a scale of 0 = emaciated to 5 = very fat with increments of 0.25 (Hervieu et al., 1991).

1) The Control group ($n = 28$; BW: 42.6 ± 0.6 kg, BC: 2.76 ± 0.07) was fed 500 g of commercial concentrate and 500 g of barley straw daily to maintain their weight in agreement with INRA requirements for a goat of 40 kg live weight. These amounts correspond to a daily intake of 0.65 milk fodder units (UFL) and 51 g of digestible protein in the intestine (PDI).

2) The Supplemented group ($n = 28$; BW 43.0 ± 0.6 kg, 2.73 BCE ± 0.06) received a high level of nutrition. These goats were fed 700 g of commercial concentrate, 500 g of barley straw and 500 g of Lucerne hay daily, which provided two times the maintenance requirements for a goat of 40 kg live weight. These amounts correspond to a daily intake of 1.2 UFL and 126.4 g of PDI.

3) The Restricted group ($n = 28$; BW 42.6 ± 0.5 kg, 2.74 BCE ± 0.05) received a low level of nutrition. They were fed with 100 g of commercial concentrate and 300 g of barley straw daily, which provided 0.3

times the maintenance requirements for a goat of 40 kg live weight. These amounts correspond to a daily intake of 0.2 UFL and 15.2 g of PDI.

The feed provided to the does was a commercial concentrate of oats (24.7%), maize (23.0%), peas (20.4%), barley (16.3%), Lucerne pellets (12.2%) and a mineral–vitamin complement (3.4%). The nutritional values of the feed were 0.94 UFL and 77 g of PDI/kg of dry matter. This concentrate was offered individually once per day to avoid the competition between females. The barley straw distributed to every animal in each group provided 0.37 UFL and 25 g of PDI/kg dry matter. In addition, the Lucerne hay provided 0.73 UFL and 120 g of PDI/kg dry matter. All animals had free access to water and mineral–vitamin blocks.

Each group of does was penned together until they were placed with the bucks. At this time, animals were housed in open barns completely isolated from those animals in the other treatment groups.

2.4. "Male effect"

On 22 March, 39 days after the end of the photoperiod treatment and 7 days after introducing the nutritional regimes, the bucks were introduced to the females until 25 April (*i.e.* for 34 days). When the males were introduced to the females, six subgroups were created according to the nutritional regime of the females (Control, Supplemented, Restricted) and the light treatment of the males (Photo, Natural) (Fig. 1).

For the bucks of the Photo group, six of the eight bucks treated (as described in Section 2.2) were selected for use in the study. During the previous week, all the bucks had been exposed for 5 min on 1 day to does in oestrus (not the experimental does), and their sexual behaviour was assessed through observation of genital sniffing, nudging and mounting attempts. The bucks of the Photo group expressing similar aspects of sexual behaviour were selected and placed with does from each nutritional group, two males per group. For the bucks of the Natural group, six of the eight bucks were chosen at random and placed with does from each nutritional group, two males per group.

Bucks were equipped with marking harnesses, placed with the experimental does and kept with them for the following 34 days (until 25 April). During this period, the bucks (Photo or Natural) in contact with the Restricted group were fed separately from the does (at the moment of the individual distribution of the concentrate to the animals). Bucks were fed adequately according to their nutritional requirements to avoid the negative effects of undernutrition.

2.5. Measurements in does

2.5.1. Body weight and body condition

The BW and BC of all does were recorded weekly during whole experiment. The BC was scored by lumbar palpation (always performed by the same handler) based on a scale of 0 = emaciated to 5 = very fat with increments of 0.25 (Hervieu et al., 1991).

2.5.2. Oestrous behaviour, ovulation and ovulation rate

From the male introduction, oestrous behaviour was recorded every day by direct visual observation of the marks from the marking harnesses (Walkden-Brown et al., 1993). The interval between the time the bucks were placed with the does and the first detected oestrous behaviour was calculated for each doe.

To monitor the ovulatory cycles of does before placement with bucks (Day 0; 22 March), blood samples were collected once per week for three consecutive weeks, and the plasma progesterone concentration was determined. Females with plasma progesterone concentrations ≤1.0 ng/mL in all samples were considered anoestrus (Chemineau et al., 1992).

After male introduction, plasma progesterone concentration was measured every 48 h for a week and thereafter twice per week until the end of the experiment to monitor the ovulatory response after male introduction. Does with plasma progesterone concentrations ≥1.0 ng/mL in at least two consecutive samples were deemed to have ovulated and to have developed a corpus luteum of normal functional duration (Chemineau et al., 1992). The date of detection of this ovulation was defined as the day when the first sample with plasma progesterone concentrations of ≥1.0 ng/mL was collected. Silent ovulations were deemed to have occurred when there was an increase in plasma progesterone ≥1.0 ng/mL detected in at least one sample but was not preceded by oestrous behaviour. The percentages of does expressing behavioural oestrus with or without ovulation and those ovulating without detection of behavioural oestrus (silent ovulation) were inferred based on the profiles for plasma progesterone concentrations.

Females in which progesterone increased to ≥1.0 ng/mL were considered to have ovulated. If this increase lasted <10 days and then increased again the doe was classified as having had a short ovulatory cycle. Females in which progesterone remained increased for at least 10 days after male exposure were classified as having entered a normal ovulatory cycle (Chemineau et al., 1984).

The occurrence of ovulation and the ovulation rate were assessed by the number of corpora lutea observed in each female by transrectal ultrasonography conducted 6 to 8 days after the detection of oestrus (Simoes et al., 2005). The procedure was performed using an Aloka SSD-

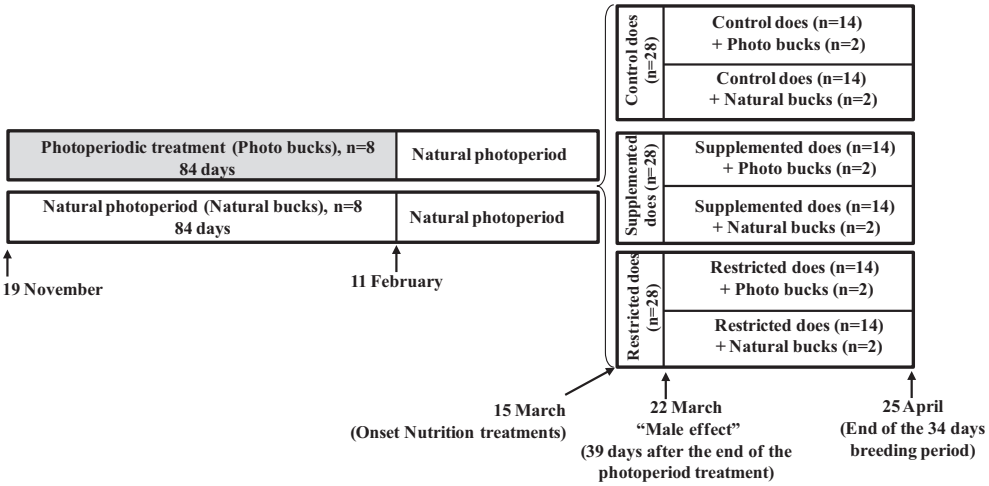

Fig. 1. Experimental design of the "male effect" of does fed daily to maintain their weight (Control), with a diet providing 2.0 (Supplemented) or 0.3 (Restricted) times the maintenance requirements, using bucks treated with artificially long days for 3 months from November to February (Photo) and untreated males (Natural).

500 (Ecotron, Madrid, Spain) ultrasound apparatus connected to a 7.5 MHz linear probe.

2.5.3. Fecundity, fertility, prolificacy and productivity

Fecundity (percentage of pregnant does/does mounted by the bucks) was determined using transrectal ultrasonography on day 45 following the detection of oestrus (Schrick et al., 1993). Fertility (percentage of does kidding/does that mated), prolificacy (number of kids born/female kidding) and productivity (number of kids born/female that mated) were also determined.

2.5.4. Plasma samples and hormone analysis

Blood was collected by jugular venepuncture in tubes containing 10 μL heparin and plasma was obtained by centrifugation at 2300 ×*g* for 30 min and stored at −20°C until analysis. Plasma samples, as described previously in Section 2.5.2, were used for measurements of progesterone, NEFAs and IGF-1 plasma concentrations.

Plasma progesterone content (ng/mL) was determined using an enzyme-linked immunoassay kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer's instructions (Andueza et al., 2014). The sensitivity of the assay was 0.2 ng/mL. Intra- and inter-assay coefficients of variation for sample pools of 0.5 ng/mL were 8.5% and 5.0% and sample pools of 1 ng/mL were 7.5% and 6.5%, respectively.

Plasma NEFAs (mg/dL) were determined immediately after sampling by spectrophotometric assay using the NEFA-HR2 kit (Wako, Chemicals GMBH, Germany). The sensitivity was 0.027 mg/dL, and the intra- and inter-assay coefficients of variation were 5.2% and 5.2%, respectively.

Plasma IGF-1 (ng/mL) was determined 2 to 3 months after sampling using the commercial Goat Insulin-Like Growth Factor kit (Cusabio, Shanghai, China), which has a sensitivity of 0.1 ng/mL, and intra- and inter-assay coefficients of variation 4.2% and 3.7%, respectively.

2.6. Buck plasma testosterone

Blood for the determination of plasma testosterone was obtained by jugular venepuncture using vacuum tubes containing 10 μL of heparin. Blood samples were collected weekly at 0900 from the beginning of the experiment (19 November until 25 April). The plasma was processed as previously described. Testosterone plasma concentrations were determined using a commercial enzyme-linked immunoassay kit (Demeditec Diagnostics, Kiel-Wellsee, Germany). The sensitivity of the assay was 0.1 ng/mL, and intra- and inter-assay coefficients of variation for sample pools of 0.2 ng/mL were 6.9% and 6.4%, and for sample pools of 6.0 ng/mL were 2.6% and 2.0%, respectively.

2.7. Statistical analyses

Data are presented in the format mean ± standard error. The values for BW, BC, progesterone, NEFA, IGF-1 in the does and testosterone in bucks were examined using an ANOVA with the repeated measures procedure and the model included fixed between-subjects experimental factors and a fixed within-subject factor for time (repeated measures), as well as the interactions between these factors. The linear model used for each parameter was as follows:

$$Y_{ijkl} = \mu + N_i + P_j + (N \times P)_{ij} + T_k + (T \times N)_{ki} + (T \times P)_{kj} + (T \times N \times P)_{kij} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the value for the dependent variable; μ is the overall mean; N_i is the fixed between-subjects effect of the nutrition of the does (i = Control, Supplemented or Restricted); P_j is the fixed between-subjects effect of photoperiod treatment of the bucks (j = Photo or Natural); T_k is the within-subject fixed effect of time; N × P, T × N, T × P, T × N × P are the interactions among these factors, and ε_{ijkl} is the residual error.

The Duncan test was used to detect differences among weeks. The mean plasma concentrations of NEFA and IGF-1 before and after buck transfer into the paddock containing does were analysed by ANOVA, using a factorial model. The linear model used for each parameter was as follows:

$$Y_{ijk} = \mu + P_i + M_j + (P \text{ x } M)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the value for the dependent variable; μ is the overall mean; is the fixed between-subjects effect of the nutrition of the does (i = Control, Supplemented or Restricted); P_j is the fixed between-subjects effect of photoperiod treatment of the bucks (j = Photo or Natural); $(N \text{ x } P)_{ij}$ is the interaction among these factors, and ε_{ijk} is the residual error.

The BW, BC, NEFA or IGF-1 plasma concentrations was determined at the moment of ovulation and at the moment of showing oestrous as the value of the data obtained near to the date of the reproductive parameter observed. They were compared by ANOVA using a factorial model. The linear model for each parameter used was the same as described for the previous ANOVA. The Duncan test was used to detect differences among experimental groups.

The variables expressed as percentages—ovulating does, does expressing oestrous behaviour and ovulating, fecundity and fertility—were analysed using multinomial logistic regression and the Fisher exact probability test for two-group comparisons as required. Ovulation rates and prolificacy were compared using the Kruskal–Wallis test for global comparisons between all experimental groups. When differences were observed, the Mann–Whitney *U* test was used to determine differences between the groups. Productivity, ovulation dates, and ovulation with oestrous behaviour were compared by ANOVA using a factorial model. The linear model for each parameter used was the same as described for the previous ANOVA. The Duncan test was used to detect differences among experimental groups.

Correlation coefficients between the progesterone, NEFA and IGF-1 plasma concentrations at different times during the experimental period were calculated by the Pearson test. All calculations were performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA). The significance was set at $P < 0.05$, and the tendency to significance was considered to be $P < 0.1$.

3. Results

3.1. Body weight, body condition, NEFAs and IGF-1 plasma concentrations at the moments of ovulation and showing oestrous

No effect of the kind of buck used to induce the "male effect" (Photo *vs* Natural bucks) or interaction between the photoperiod treatment of the bucks and nutritional treatment of the does was observed on none of the studied parameters ($P > 0.05$) (Table 1).

At the time of ovulation, the BW and the BC were higher in the Supplemented and Control groups than in the Restricted females ($P < 0.001$). There was no statistically significant difference between the Supplemented and Control groups. Concentrations of NEFAs were higher in the Restricted females than in the other groups ($P < 0.001$). The IGF-1 plasma concentrations were slightly higher in the Supplemented group than in the Control group ($P = 0.069$) (Table 1).

When showing oestrus, the BW of the Supplemented group was higher ($P < 0.05$) than that of the does showing oestrous in the Restricted group, meanwhile BW of Control does did not differ from the BW of the Restricted group ($P > 0.05$). No differences between the BC of the groups were observed ($P > 0.05$). The NEFAs plasma concentrations were higher in the Restricted females than in the other groups ($P < 0.001$). The IGF-1 plasma concentrations were higher in the Supplemented group than in the Control group ($P < 0.05$) (Table 1).

3.2. Evolution of NEFAs and IGF-1 plasma concentrations and their relation to reproductive events

For the NEFAs plasma concentrations, a clear effect of time was

Table 1
Body weight, body condition (BC), NEFAs and IGF-1 plasma concentrations at the moment of ovulation and at the moment of showing oestrous of does submitted to the "male effect" using bucks treated with artificially long days for 3 months from November to February (Photoperiod) or untreated males (Natural) and fed daily to maintain their weight (Control), with a diet providing 2.0 (Supplemented) or 0.3 (Restricted) times the maintenance requirements.

Variables	Feeding level			Kind of bucks		Significance[1]		
	Control	Supplemented	Restricted	Natural	Photoperiod	Feeding level	Kind of bucks	Interaction
	$n = 28$	$n = 28$	$n = 28$	$n = 42$	$n = 42$			
Body weight at ovulation	43.0 ± 0.7a	44.1 ± 0.7a	40.3 ± 0.6b	41.8 ± 0.7	43.1 ± 0.6	***	NS	NS
BC at ovulation	2.74 ± 0.06a	2.87 ± 0.06a	2.52 ± 0.03b	2.71 ± 0.06	2.72 ± 0.04	***	NS	NS
NEFAs at ovulation	9.49 ± 0.97b	8.97 ± 1.20b	19.44 ± 1.03a	13.64 ± 1.24	11.52 ± 1.08	***	NS	NS
IGF-1 at ovulation	32.32 ± 7.56b	68.54 ± 15.85a	40.58 ± 8.26ab	46.43 ± 8.16	48.87 ± 10.80	0.069	NS	NS
Body weight at oestrous	42.7 ± 0.8ab	43.5 ± 0.8a	40.7 ± 0.6b	41.9 ± 0.6	42.8 ± 0.6	*	NS	NS
BC at oestrous	2.77 ± 0.07	2.82 ± 0.07	2.68 ± 0.04	2.77 ± 0.06	2.76 ± 0.05	NS	NS	NS
NEFAs at oestrous	8.83 ± 0.80b	6.31 ± 0.58b	22.79 ± 2.07a	13.36 ± 2.00	11.14 ± 1.13	***	NS	NS
IGF-1 at oestrous	29.96 ± 5.51b	62.07 ± 8.89a	42.94 ± 9.09ab	47.04 ± 6.90	43.03 ± 6.63	*	NS	NS

[1] NS – not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters (lower case) in the same row reflect significant differences at $P < 0.05$.

observed ($P < 0.001$). Moreover, an interaction between time × nutritional treatment of the does was observed ($P < 0.001$) (Fig. 2). This interaction showed that before the nutritional treatments began all groups showed similar NEFAs plasma concentrations, but after the first week they were applied, the NEFAs plasma concentrations of the group Restricted increased while they were maintained in the other two groups. Furthermore, the nutritional regime had a significant effect on the NEFAs plasma concentrations ($P < 0.001$), with higher plasma concentrations in the Restricted group (16.95 ± 0.41 mg/dL) than in the Control (8.36 ± 0.26 mg/dL) or Supplemented (7.02 ± 0.28 mg/dL) groups. From the moment the bucks were introduced to the groups of females, the NEFAs plasma concentrations of the females in contact with the Natural bucks were higher than in the females in contact with Photo bucks (11.38 ± 0.35 mg/dL *vs* 10.18 ± 0.32 mg/dL, respectively, $P < 0.05$). No interaction between the photoperiod treatment of bucks x nutritional treatment of the does was observed on NEFAs plasma concentrations ($P > 0.05$).

For the IGF-1 plasma concentrations, a clear effect of time was observed ($P < 0.001$). Moreover, interaction time × nutritional treatment of the does was observed ($P < 0.01$) (Fig. 3). This interaction showed that before the nutritional treatments began, all groups showed similar IGF-1 plasma concentration. However, after the experimental nutritional regimen were applied, the IGF-1 plasma concentrations of the Supplemented group increased and were maintained for around 10 days. After that, the IGF-1 plasma concentrations of all the groups increased with some differences among groups, until 18 of April when the differences disappeared until the end of the experiment (Fig. 3). Furthermore, the nutritional treatment tended ($P = 0.061$) to affect IGF-1 plasma concentrations, with higher plasma concentrations in the

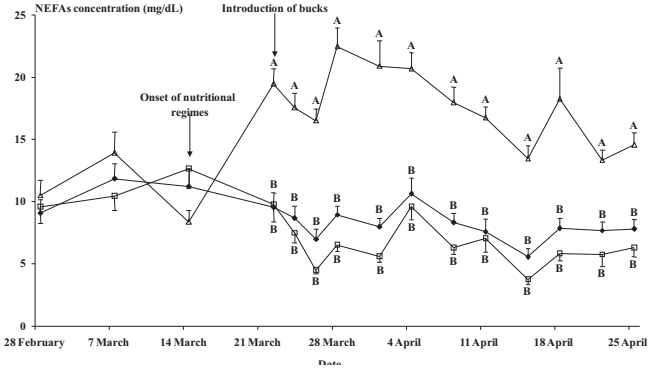

Fig. 2. Change in NEFAs (mg/dL, Mean ± SEM) in does submitted to the "male effect" fed daily to maintain their weight (Control, ◆), with a diet providing 2.0 (Supplemented, □) or 0.3 (Restricted, Δ) times the maintenance requirements. Different letters in the same week denote differences between treatments: A, B: ($P < 0.001$).

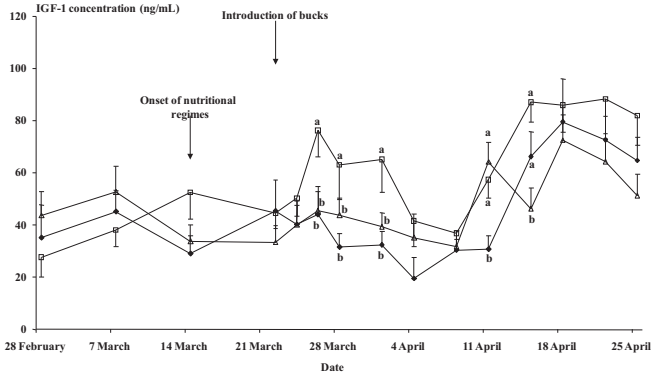

Fig. 3. Change in IGF-1 concentration (ng/mL, Mean ± SEM) in does submitted to the "male effect" fed daily to maintain their weight (Control, ◆), with a diet providing 2.0 (Supplemented, □) or 0.3 (Restricted, Δ) times the maintenance requirements. Different letters in the same week denote differences between treatments: a, b: ($P < 0.05$).

Supplemented group (63.91 ± 2.86 ng/mL) than in the Control (45.14 ± 2.61 ng/mL) or Restricted (46.26 ± 2.20 ng/mL) groups. No effect of the photoperiod treatment of the used bucks or interaction between photoperiod treatment of the bucks x nutritional treatment of the does was observed on IGF-1 plasma concentrations ($P > 0.05$).

The IGF-1 plasma concentrations showed a profile very similar to the progesterone plasma concentrations. The correlation coefficient between IGF-1 and progesterone plasma concentrations over the whole experiment, from the introduction of the males, was 0.163 ($P < 0.001$). The IGF-1 plasma concentrations increased in the Supplemented groups compared to the other nutritional groups after the introduction of the males from 25 to 29 March (Fig. 3). During this period, the correlation coefficient of the IGF-1 and progesterone plasma concentrations for the Supplemented groups was 0.270 ($P < 0.05$). Thereafter, from 8 April to the end of the experiment, when the higher ovarian response was observed, the correlation was 0.113 ($P < 0.01$). When we analyse all of the animals from the moment of the male introduction, an effect can be observed, with higher IGF-1 plasma concentrations in females showing ovulation than in females without ovulation ($P < 0.001$) (Fig. 4). This effect was maintained when comparing the IGF-1 plasma concentrations of the females showing oestrous *vs* females without oestrous, pregnant females *vs* females without pregnancy, and females giving birth *vs* females did not get pregnant and did not get birth (Fig. 4).

3.3. Doe reproductive response

No interaction was observed between the nutritional treatment of the females (Control, Supplemented, Restricted) and the treatment of the

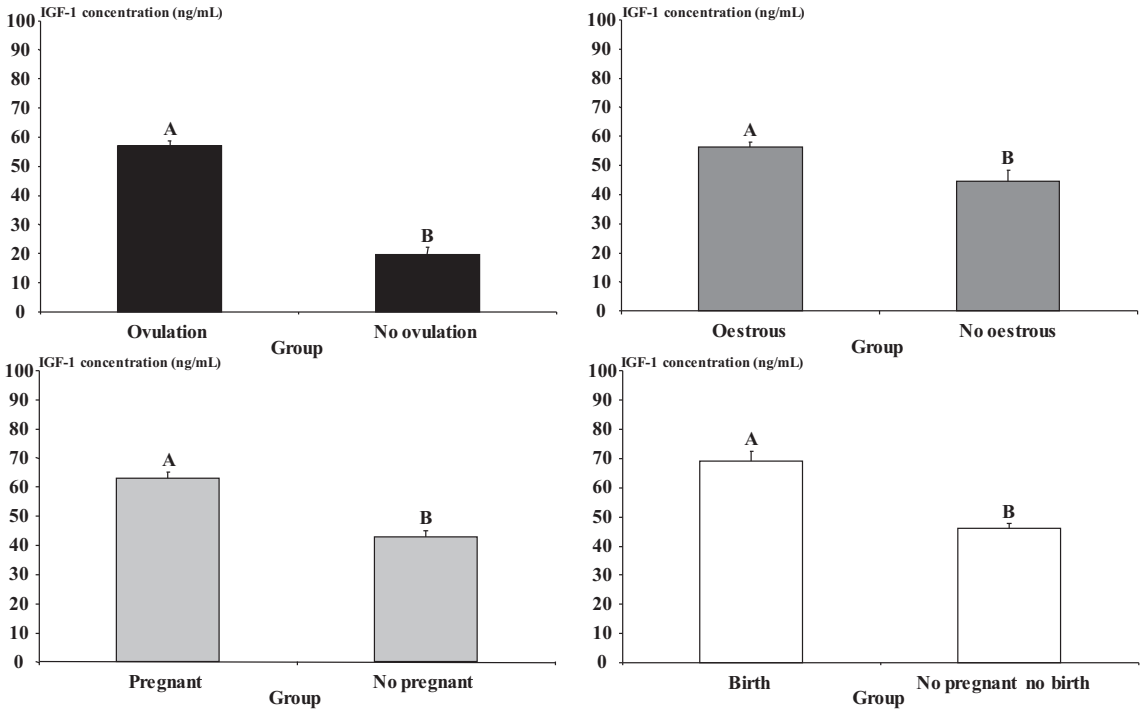

Fig. 4. Mean IGF-1 concentrations (ng/mL, Mean ± SEM) in does showing ovulation *vs* does without ovulation (■), does showing oestrous *vs* does without oestrous (■), pregnant does *vs* does without pregnancy (■) and does with successful births *vs* does did not get pregnant and did not get birth (□). The does were fed daily to maintain their weight (Control), with a diet providing 2.0 (Supplemented) or 0.3 (Restricted) times the maintenance requirements and were submitted to the "male effect" using artificially treated males for long days for 3 months from November to February, or untreated males. Different letters in the same week denote differences between treatments: A, B: ($P < 0.001$).

males (Photo, Natural) except for the interval between the introduction of the males and the first ovulation ($P < 0.05$). The interval between the introduction of the males and the first ovulation was longer in the Restricted–Natural pairing than in the Restricted–Photoperiod pairing, which was similar to the other groups (Fig. 5).

The nutritional treatment of the females affected the ovulation rate ($P < 0.001$), the fertility ($P < 0.05$) and the productivity ($P < 0.01$), which were higher in the Supplemented group than in the Restricted group (Table 2). Moreover, the effect of nutrition on fecundity was close to significance ($P = 0.077$). The photostimulation of the males increased the percentage of females that showed oestrous and ovulation after male introduction ($P < 0.05$) (Table 2).

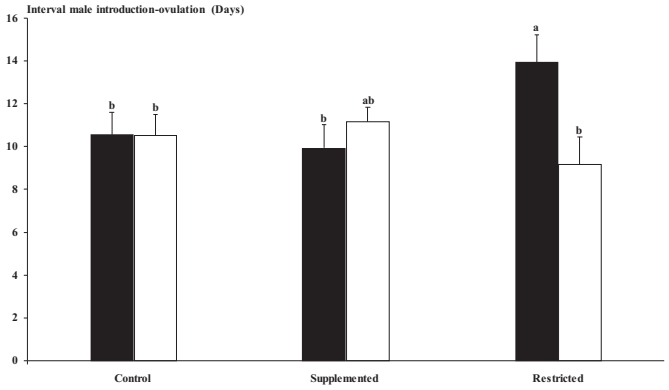

Fig. 5. Interval (days, Mean ± SEM) between the introduction of the bucks and the ovulation of the does the does fed daily to maintain their weight (Control), and 2.0 (Supplemented) or 0.3 (Restricted) times the maintenance requirements and submitted to the "male effect" using bucks treated with artificially long days for 3 months from November to February (Photo group, □) and untreated males (Natural group, ■). Different letters differ significantly: a, b: ($P < 0.05$)."

No differences between photoperiod treatment of the used bucks or the nutritional regimes of the females was observed on the number of short cycles showed by the females (33% and 31% for Natural and Photoperiod groups, respectively $P > 0.05$; 39%, 29%, and 29% for the Control, Supplemented and Restricted groups, respectively; $P > 0.05$).

3.4. Testosterone plasma concentrations

Time had a distinguishable effect on the plasma testosterone plasma concentration ($P < 0.01$) as the interaction time × photoperiod treatment of the bucks ($P < 0.01$). However, time × nutritional treatment of the does or time × nutritional treatment of the does × photoperiod treatment of the bucks were not statistically different ($P > 0.05$). The bucks of the Photo group had greater testosterone plasma concentrations than the bucks of the Natural group from 4 March until 18 March ($P < 0.01$) (Fig. 6), when bucks were placed in contact with does for induction of the "male effect". A sudden rise in testosterone plasma concentrations was observed in the Natural group on 25 March, precisely when the males were used for the male effect.

4. Discussion

The results of the present study indicate that at Mediterranean latitudes, although the sexual activity of the male is essential to induce the "male effect", the nutritional status of the female is very important. No interaction between both sources of variation was observed, and therefore, we cannot counterbalance the negative effect of one of them by improving the other one. The absence of interaction between both sources of variation in the most part of studied variables could be due to the used number of animals, that perhaps needs to be higher, and thus more important practical implications could be achieved.

When we analyse the effect of the photostimulation of the males on their reproductive performances, only the percentage of females showing oestrus and ovulating was improved by the kind of males used.

Table 2
Reproductive response, of does submitted to the "male effect" using bucks treated with artificially long days for 3 months from November to February (Photoperiod) or untreated males (Natural) and fed daily to maintain their weight (Control), with a diet providing 2.0 (Supplemented) or 0.3 (Restricted) times the maintenance requirements.

Variables	Feeding level			Kind of bucks		Significance[1]		
	Control	Supplemented	Restricted	Natural	Photoperiod	Feeding level	Kind of bucks	Interaction
	$n = 28$	$n = 28$	$n = 28$	$n = 42$	$n = 42$			
Ovulating does (%)	89	96	89	88	95	NS	NS	NS
Does showing silent ovulations (%)	46	50	32	36	50	NS	NS	NS
Does showing short cycles (%)	50	50	32	36	52	NS	NS	NS
Interval introduction of males-normal ovulation (days)	10.52 ± 0.71	10.56 ± 0.64	11.43 ± 1.02	11.37 ± 0.71	10.33 ± 0.57	NS	NS	*
Does in oestrus and ovulating (%)	78	89	75	71	90	NS	*	NS
Interval introduction of males and oestrus (days)	6.58 ± 0.57	6.13 ± 0.47	7.76 ± 1.21	7.28 ± 0.66	6.35 ± 0.61	NS	NS	NS
Ovulation rate	1.58 ± 0.14A	1.77 ± 0.13A	1.05 ± 0.05B	1.38 ± 0.10	1.61 ± 0.11	***	NS	NS
Fecundity (%)	50ab	71a	43b	52	57	0.077	NS	NS
Fertility (%)	45b	76a	29b	57	47	*	NS	NS
Prolificacy	1.60 ± 0.16	1.47 ± 0.12	1.33 ± 0.21	1.53 ± 0.13	1.44 ± 0.12	NS	NS	NS
Productivity	0.57 ± 0.16b	1.00 ± 0.15a	0.29 ± 0.11b	0.62 ± 0.17	0.62 ± 0.12	**	NS	NS

[1] NS – not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Different letters (lower case) in the same row reflect significant differences at $P < 0.05$. Different letters (capital letter) in the same row reflect significant differences at $P < 0.01$.

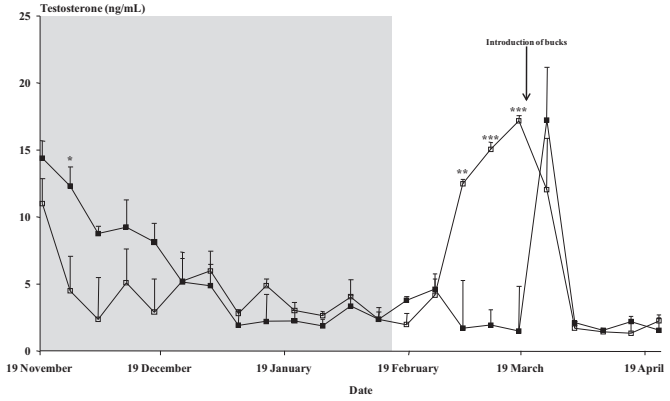

Fig. 6. Plasma testosterone concentration (Mean ± SEM, ng/mL) of bucks treated with artificially long days for 3 months from November to February (Photo group: □, $n = 6$) and untreated males (Natural group: ■, $n = 6$); The grey area indicates the time of the photoperiod treatment. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

This confirms results suggesting that buck reproductive state is a key factor for the induction of reproductive responses in a large percentage of does. Doe response to bucks is more profound when sexually active males are used (Flores et al., 2000; Delgadillo et al., 2002; Veliz et al., 2002). In the present experiment, as in other of our group (Zarazaga et al., 2019a), we chose bucks with similar reproductive behaviour, but all of them showed similar and intense sexual behaviour.

The ovulation rate was lower in the restricted female group while fertility and productivity were higher in the supplemented female group. The Supplemented nutritional group showed higher BW and BC at the moment of ovulation and higher BW but similar BC when showing oestrous than the Restricted group. This last fact was because the females that showed oestrous in the Restricted group were the females with higher BC, even though the groups were balanced at the onset of the experiment. These findings agree with observations made by Veliz et al. (2006), who reported that irrespective of their BC, heavier female goats responded better to the "male effect" than lighter animals. However, that study was focused on animals with a stable BW and BC, whereas the present experiment examines animals provided with supplementation or acute nutritional restriction respond to the "male effect". In this way, the results of the current study agree with previous results obtained by our group (Gallego-Calvo et al., 2014b, 2015a, 2018; Zarazaga et al., 2017) in which a better reproductive response was observed with long-term supplemented nutrition. According to our previous results, it was expected that the groups provided with a higher level of nutrition would show better results that, could be explained by a more intense sexual interactions between males and females observed in these groups (Gallego-Calvo et al., 2015a, 2018; Zarazaga et al., 2017).

Moreover, these results agree with previous results obtained by De Santiago-Miramontes et al. (2008). These authors observed an increase in the ovulation rate of goats supplemented for 7 days and submitted to the "male effect" in spring. Similarly, Fitz-Rodríguez et al. (2009) observed an increase in the ovulation rate of feed-supplemented females, starting the supplementation at the same time as the introduction of the males for 7 days. They also saw an increased pregnancy rate in females supplemented for 14 or 28 days starting this supplementation, 9 days after males were introduced. However, in both experiments, the only males used to induce the "male effect" were photostimulated. In our experiment, we tried to determine if nutritional supplementation resulted in better results for females submitted to the "male effect" using males showing springtime sexual activity or if the photostimulated bucks can counterbalance the negative effect of an acute nutritional restriction. Our results demonstrate one factor cannot counterbalance the negative effect of the other one.

The interaction between kind of males and nutrition on the interval between male introduction and the moment of ovulation determined that for the Restricted females, this interval is reduced when photostimulated bucks are used compared to the interval when the bucks used have been submitted to the natural photoperiod. This result could be explained by a higher sexual interactions initiated by the photostimulated bucks (Zarazaga et al., 2019b), which induced a quicker reproductive response in females with a negative nutritional status. However, in the other groups, there were no statistically significant differences in the responses of the females grouped with Photo or Natural bucks.

A possible explanation for the enhanced reproductive results could be due to the nutritional parameters. Both glucose and insulin are involved in energy homeostasis. We did not analyse glucose or insulin plasma concentrations because, in previous experiments (Gallego-Calvo et al., 2015a, 2018), we observed that in both cases, for the duration of the experiment, the animals receiving supplemental nutrition showed higher glucose and insulin concentrations. Therefore, in the present

experiment, it was expected that the glucose and insulin concentrations would be higher in the supplemented females throughout the entire experiment.

The NEFAs concentrations were higher from the onset of the nutritional regimes, indicating that body fat reserves were mobilised in the nutritionally restricted females. As in our other experiments (Gallego-Calvo et al., 2015a, 2018), a clear decrease in the plasma NEFAs concentration occurred after male introduction, especially when the progesterone concentrations increased. However, in the present experiment the NEFAs concentrations were higher in the females in contact with the Photo bucks that showed slightly higher ovulatory response, that could be explained because in that experiments the nutritional regimes were established to long term effect. Moreover, the correlation coefficient between NEFAs and progesterone concentrations from the introduction of the males was −0.076 ($P < 0.05$), and from 1 to 11 April, when the higher ovulatory response was observed, the correlation was −0.108 ($P < 0.05$). In cows, it has been reported that NEFAs affect reproductive performance by influencing ovarian follicle growth, progesterone production, embryo viability and maintenance of pregnancy (Santos et al., 2008). The NEFAs concentrations were higher in the Restricted females than the other two nutritional groups at the moment of ovulation and oestrus. This difference could explain the lower ovulation rate of the Restricted in comparison to the other nutritional groups.

The IGF-1 concentrations showed an evolution similar to the progesterone concentrations. However, the increase of IGF-1 concentrations in the Supplemented group after male introduction cannot be explained by the short cycles because there were similar percentages in all experimental groups. It has been hypothesised in several reports that the hypothalamo-pituitary axis receives reproduction-promoting metabolic signals *via* IGF-1 of peripheral origin, and Flores et al. (2014) found that does with greater food intake had greater IGF-1 concentrations than control animals. Moreover, when there is an increase in IGF-1, the production of steroids increases and ovarian follicle growth is enhanced (Spicer and Echternkamp, 1995). Martínez et al. (2011) have suggested that the oestrous response and pregnancy rate can be improved *via* the effect of IGF-1 on follicle development. The increase in IGF-1 concentrations after the placement of bucks with does suggests this growth factor may be involved in some reproductive responses that occur due to the male effect. Moreover, when the IGF-1 concentrations were analysed with respect to the reproductive response, higher IGF-1 concentrations were observed in females responding to the "male effect". High plasma IGF-1 has been described as a useful predictor of reproductive success in cattle (Doyle et al., 2019). Taylor et al. (2004) reported that cows with plasma IGF-1 values greater than 50 ng/mL at first servicing exhibited a five-fold increase in the likelihood of conception. Moyes et al. (2003) found that plasma IGF-1 concentrations in pregnant cows were higher after conception than those of non-pregnant cows and stayed high throughout the pregnancy, similar to the results observed in the present experiment after the 11 April.

5. Conclusions

When goats are in Mediterranean latitudes, buck reproductive state is an important factor to induce a higher percentage of females showing oestrus and ovulating in response to the "male effect". However, the effects of the nutritional status of the females on the "male effect" must not be discarded, whether it is an acute restriction or an acute supplementation. Both sources of variation act independently; therefore, one cannot counterbalance the negative effect of the other. An acute restriction starting 7 days before male introduction induces a reduction in the ovulation rate, fertility and productivity compared to an acute supplementation. The IGF-1 concentrations were higher independent of the nutritional group in the females that showed response to the "male effect".

Funding

This study was funded by Grant AGL2016-75848-R from MINECO-AEI-FEDER (Spain).

Acknowledgements

The authors wish to thank the farm staff of Huelva University for their technical support. Funding for open access charge: Universidad de Huelva/CBUA.

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