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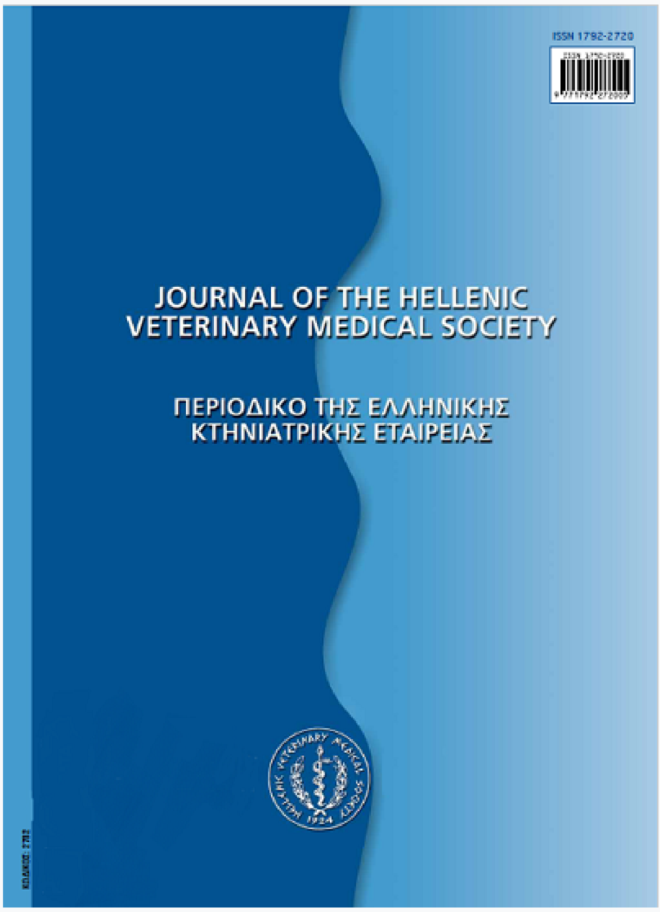

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Effects of testosterone administration and feeding level on reproductive activity in sexually inactive goat bucks

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ABSTRACT. The aim of this study was to investigate the effect of the administration of testosterone to a well-fed or underfed sexually inactive goat bucks on their sexual behavior and semen characteristics. Twenty sexually experienced mixed-breed goat bucks (1.5 years of age) were randomly assigned to one of four treatments for a period of 71 days. Treatments consisted of diets with amount of nutrients to meet 1.0 times the nutrient maintenance requirements with the application of 25 mg testosterone every third day during 21 d (treatment 1T) or saline (treatment 1C). A third and fourth treatments received a diet 1/2 times the nutrient maintenance requirements with testosterone (1/2T) or saline (1/2C). Bucks on 1T and 1C had higher body weight, body condition score and scrotal circumference compared to 1/2T and 1/2C ($P<0.05$). Also, bucks on 1T and 1/2T had higher sexual odor compared to 1C and 1/2C ($P<0.05$). Mean serum testosterone concentrations were highest in 1T (4.78 ± 6.78 ng/ml) and lowest in 1/2C (0.56 ± 0.96 ng/ml; $P<0.01$). Semen volume, sperm concentration, and mass motility were not affected by treatments, but sperm progressive motility was lower ($P<0.05$) in 1/2C (46%) than the other treatments (52 to 57%). Courtship traits and mounts were more frequent ($P<0.05$) in 1T compared with the other treatments. Likewise, bucks in the 1T group had the shortest latency to first mount (82 seconds) compared to other treatments (110 to 164 seconds). These results indicate that testosterone administration to well-fed sexually inactive bucks provokes clearly defined sexual activity during the non-breeding season. However, these benefits are overridden by underfeeding goat bucks.

Keywords: Semen characteristics; Flehmen; Sexual odor; Sperm motility; Scrotal circumference

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INTRODUCTION

Most goats in developing countries are exploited on pastures, managed under traditional extensive systems. Grazing goat bucks need an even and continuous supply of feed for their maintenance, and other productive processes, such as reproduction. Goat bucks on arid and semi-arid rangelands subsist solely on native vegetation throughout the year, which implies successive periods of shortages and surfeits of forage. Under these circumstances nutrient supply and body energy reserves vary widely during the year, affecting the reproductive activity of goat bucks (Zarazaga et al., 2009). Another consequence of underfeeding goat bucks is a significant detrimental effect on the testicular development and semen quality in these animals (Almeida et al., 2007; Zarazaga et al., 2009).

Another important factor affecting buck sexual drive is the season of the year. In temperate zones, libido, fertility, and semen quality and volume are better in late summer and fall (Roca et al., 1992; Karagiannidis et al., 2000; Barkawi et al., 2006). As the daylength gets longer, sperm concentration is lower, whereas it is noticed that the percentage of morphologically abnormal spermatozoa may be increased. (Barkawi et al., 2006; Ramadan et al., 2009). Recent reports indicate that the administration of testosterone to bucks during the non-breeding period (Spring) elicits a strong sexual behavior in goat bucks (Luna-Orozco et al., 2012; Ángel-García et al., 2015a), which in turn induces ovulation in anestrous goats. However, it is unknown if the effect of exogenous testosterone is modified by the nutritional status of bucks. Therefore, the objective of this study was to determine whether nutritional status combined with the administration of testosterone influence the sexual behavior and semen quality of goat bucks during the non-breeding season.

MATERIAL AND METHODS

Study area and animal housing and management

The Autonomous Agrarian University Antonio Narro ethics committee approved all the experimental work. This study was carried out in northern Mexico (25° N, mean annual temperature 23.5°C). The climate is semi-arid, with hot spring and summer and mild winters. Most of the precipitation occurs during the summer and fall.

A total of 20 mature mixed-breed (native x dairy breeds) goat bucks, initially averaging 41 ± 3.7 kg body weight (BW) and 2.9 ± 0.3 body condition score (5-point scale), with similar testis size at the start of the study were used. Prior to the experiment, the goat bucks were kept under rangeland conditions. Then the goat bucks were housed in a 4 x 10 m outdoor roofed pens with dirt floors, under ambient temperature and lighting. The trial was carried out over a period of 71 days (February 3 to April 15, 2014).

The bucks were randomly allotted to 4 experimental groups: 1.0 x the maintenance nutrient requirements with (1T, n= 5) or without (1C, n=5) administration of testosterone, and 0.5 x the maintenance nutrient requirements with (1/2T, n= 5) or without (1/2C, n=5) testosterone. The goat bucks on the 1.0 x the maintenance nutrient requirements diet were offered 500 g day^{-1} of alfalfa hay, 1100 g of oat hay, 200 g of molasses and 300 g day^{-1} of concentrate (14% crude protein). Those on the 0.5 diet were offered half of the feed previously described. The different nutritional planes were based on NRC (2007) and the goat bucks were provided with these diets for 71 days. Water and salt-mixed trace minerals were offered ad libitum. From March 24 to April 14, bucks on the 1T and 1/2T received 25 mg testosterone (Testosterona 50®, Brovel Laboratory, Mexico D.F., Mexico) i.m. every third day. Bucks on the 1C and 1/2C groups received saline injections.

Collection of data

The change in body weight and body condition score on each treatment was recorded weekly. Body condition score (BCS) was assessed by palpating the muscle mass at the lumbar region of goat bucks, and values were assigned based on a 1–5 points scale (1= very thin, 5= very fat; Detweiler et al., 2008). Scrotal circumference measurements were recorded weekly on all goat bucks throughout the experimental period. Measurements were done by using a flexible measuring tape placed in the widest part of both testicles.

The intensity of the odor was recorded weekly for each buck, by smelling the dorsum of the neck approximately 15 cm behind the caudomedial border of each horn base (Walkden-Brown et al., 1999). The score scale used was from 0 (neutral odor, not different from a female) to 3 (strong male odor).

Ejaculates were successfully collected on all bucks during 5 consecutive weeks, at the middle of the supplementation period (5 weeks after the beginning of the feed supplementation), by means of an artificial vagina. The total volume of the ejaculate was measured using a calibrated test tube. Sperm mass motility of undiluted semen was subjectively estimated by a light microscope (x 400) at 37° C. Three different microscopic fields for each semen sample were observed and the mean of these estimations gave the motility score using a scale from 0 (no activity) to 5 (rapid swirling motion). Sperm concentration was evaluated by a spectrophotometer calibrated for goat bucks (Minitube, Tiefenbach, Germany) following the protocol described by Prathalingam et al. (2006). Furthermore, progressive motility was assessed by a light microscope (x400) at 37ºC.

On 14 and 15 of April of the experimental period, a sexual behavior test was conducted. Goat bucks from all experimental groups were individually exposed to one unrestrained estrogenized female for 20 min in an isolated pen. The sexual behaviors were recorded uninterruptedly during the test period by an observer positioned outside the pen at a distance of approximately 2.5 m. Each buck was used in only one test per day. Two females were estrogenized by administering 2 mg estradiol cypionate i.m. every third day (ECP®, Lab. Pharmacia & Upjohn, Mexico) during 3 consecutive weeks. The behavioral components recorded were: latency to first mount, number of vocalizations, anogenital sniffing, flehmen response, approximations to females, mount attempts, mounts without ejaculation, mounts with exposed penis, mounts with ejaculation (buck thrusting forward and leaping off the ground, propelling the doe forwards). Total serum testosterone was assessed using an ELISA assay according to the kit protocol (Neogen Corporation #402510; Lexington, KY, USA). Ten blood samples (5 ml) were collected weekly during the study period into Vacutainers (Corvac Kendall Health Care, St. Louis, USA) from the jugular vein of each goat buck, and allowed to clot at room temperature during 30 min. Serum was separated by centrifugation at 5000 g for 10 min, immediately transferred to polypropylene micro tubes (Axygen Scientific, Union City, USA) and stored at -20°C until further analysis.

Statistical analyses

The Shapiro Wilk W-test was used to analyze data for normality. Heterogeneity of variance was found for courtship behaviors, mount attempts, and mounts with ejaculations using Bartlett's Box F-test specifying this option in the MEANS Statement in SAS (SAS Inst. Inc., Cary, NC). The data for courtship behaviors, mounts, and mounts with ejaculations were transformed to the reciprocal of square root of X + 1, log of X + 1, and square root of X + 0.1, respectively, which stabilized the variances among goat bucks for the fixed effects. Data of sperm motility were arcsine transferred prior to analysis. The effect of feeding level and testosterone administration on goat buck body weights and BCS was determined using mixed-model methodology using PROC MIXED in SAS. Within these analyzes, goat buck was treated as a random effect and feeding level and administration of testosterone were included as fixed effects in the model.

Odor intensity data were analyzed using the Wilcoxon non-parametric tests (NPAR1WAY procedure; SAS) to test for differences between groups of goat bucks. Plasma testosterone data were analyzed using mixed model procedures of SAS (SAS Inst. Inc., Cary, NC) for specific repeated measures, considering 10 blood collection times. Data for testosterone showed heterogeneous variance using Bartlett's Box F-Test in SAS. To normalize variances among goat bucks for fixed effects, testosterone values were transformed to the inverse square root. Testosterone means were changed back to their original units after analysis.

A total of 5 ejaculates, each from the 20 goat bucks, were examined by repeated measures ANOVA using a general linear model (PROC GLM of SAS) procedure. The statistical model included the effects of feed level, testosterone administration and their first-order interactions on sperm characteristics. Differences were considered to be statistically significant at $P<0.05$.

RESULTS

The mean values of body weight, body condition score, sexual odor intensity and scrotal circumference throughout the study are presented in Table 1. Body weight was lower (P<0.05) for underfed groups than for well-fed goat bucks. Likewise, BCS increased when animals were adequately fed compared with the underfed goat bucks. The intensity of sexual odor was greater (P<0.05) in goat bucks treated with testosterone, regardless of the feeding level, compared to bucks non-receiving testosterone. Bucks fed adequate levels of nutrients had larger mean scrotal circumference (P<0.05) after 70 d of the trial (Table 1). No feed level x testosterone treatment interactions were detected for the aforementioned variables.

A beneficial effect of improved feeding and exogenous testosterone administration was observed on serum testosterone levels. Serum testosterone levels of group 1T were 5.7 times higher(P<0.05) than 1C bucks (Table 2). Likewise, serum testosterone levels in 1T bucks were about twice that observed in 1/2T bucks (P<0.05). The feed level x testosterone treatment interaction was important (P<0.05), with better response of well-fed bucks to the testosterone treatment compared to underfed bucks.

Data revealed that no significant differences existed between the four groups for semen volume, sperm concentration and mass motility. Underfed goat bucks treated with testosterone had a significant (P<0.05) decline in sperm progressive motility as compared to all other groups. The main effect of feeding level and the feeding level x testosterone treatment interaction were found to be significant at statistical level (P<0.05; Table 2).

The total numbers of sexual behaviors of each buck on the tests with estrogenized does are given in Table 3. There was a greater frequency (P<0.01) in the act of the male sniffing the anogenital region and urine of does in well-fed testosterone-treated bucks, compared with bucks in other groups. Moreover, during the season of sexual inactivity, well-fed and testosterone treated bucks emitted a greater (P<0.05) number of vocalizations than bucks in the other groups. However, higher plane of nutrition and exogenous testosterone did not increase the flehmen reaction of bucks. Likewise, the flehmen reaction was expressed at similar frequencies in well-fed and underfed bucks.

Approximations to does were performed more often (P<0.05) by the well-fed testosterone-treated bucks compared with all other groups. There was no difference in approximations neither between well-fed and underfed bucks nor between bucks treated with or without testosterone. Regarding the act of mounts attempts, there was a greater frequency in bucks treated with testosterone compared to bucks not treated with testosterone and no effect of nutritional status was observed. However, neither feeding level nor testosterone treatment influenced number of complete mounts. Both mounts with the penis exposed and mounts with ejaculation were greater (P<0.05)

Table 1: Body traits, sexual odor intensity and scrotal circumference of well-fed or underfed mixed-breed goat bucks treated with exogenous testosterone or saline during the non-breeding season. Values are means ± standard deviation.

Item*	1C	1T	1/2C	1/2T
Body weight, kg	42.8 ± 9.9^{a}	45.8 ± 3.2^{b}	39.1 ± 5.6^{c}	39.7 ± 8.9^{c}
Body condition score*	2.4 ± 0.4^{a}	2.5 ± 0.3^{a}	2.1 ± 0.4^{b}	2.2 ± 0.6^{b}
Sexual odor intensity**	0.15 ± 0.23^{a}	0.29 ± 0.38^{b}	0.12 ± 0.21^{a}	0.26 ± 0.34^{b}
Scrotal circumference, cm	26.8 ± 2.0^{a}	26.9± 1.2^{a}	25.1 ± 1.3^{b}	1.9 ± 2.7^{b}

1C=100% nutrient requirements for maintenance; 1T= 100% nutrient requirements for maintenance plus testosterone; 1/2C= 50% nutrient requirements for maintenance; 1/2T= 50% nutrient requirements for maintenance plus testosterone.
**5-point scale; **0 to 3-point scale.*
For all variables, no feeding level x testosterone treatment interactions (P>0.05) were found.
^{a,b,c}*Means in the same raw with different superscript differ (P<0.05).*

in group 1T than in the other groups. Latency to first mount of 1T goat bucks was half the time recorded from the 1C goat bucks.

DISCUSSION

At the beginning of the study (spring; increasing daylength), the goat bucks were sexually quiescent with low serum testosterone, no male sexual odor, and few signs of peripheral androgen stimulation. Underfed animals under these conditions presented retardation in body development and scrotal circumference shared this retardation. This study confirms earlier reports that have indicated a significant association between plane of nutrition, body weight and scrotal circumference (Mellado et al., 2012; Ghorbankhani et al., 2015). The fact that testosterone administration did not affect scrotal circumference indicates that changes in testicular size could be induced solely by diet. The lack of nutrients was apparently associated with changes in gonadotrophin secretion, suggesting that there is an effect of nutritional status on the hypothalamic-pituitary axis regarding the testicular development of mixed-breed goat bucks (Martin et al., 1994; Blache et al., 2000). Scrotal circumference was enhanced with the higher feeding level, which is contrary to findings of Tufarelli et al. (2011) and Bielli et al. (1999), who found no significant effect of improved diets on scrotal dimensions. However, findings of the present study are consistent with data of Hötzel et al. (2003) and Fernández et al. (2004). These differences can mainly be attributed to greater amounts of subcutaneous fat in the scrotal skin in goat bucks fed the 100% maintenance diet, as it has been observed by Fourie et al. (2004).

Sexual odor intensity was strongly affected by the administration of testosterone, regardless of the nutritional status of goat bucks. This finding can be ascribed to the fact that the primer pheromone responsible for the male effect is produced in the sebaceous glands (primarily in the cornual gland; Van Lancker et al., 2005) under the control of testosterone (Iwata et al., 2000). Thus, apparently, the sebaceous glands developed during the course of the testosterone treatment inducing the pheromone (odor) production.

The serum testosterone levels were clearly much higher in the well-fed testosterone-treated bucks, confirming the findings of previous studies showing that serum testosterone levels are three times higher in testosterone-treated bucks compared to control during the non-breeding season (Ángel-García et al., 2015b). The fact that testosterone levels were lower in underfed bucks compared to the well-fed bucks indicates that diets significantly affected the blood testosterone patterns. The interaction between feeding regime and testosterone treatment further shows that the energy body reserves alters the serum testosterone levels of bucks during the non-breeding season. Other studies have demonstrated that testosterone secretion in goat bucks is influenced by feed (Al-Sobayil et al., 2008). Therefore, it could be hypothesized that these differences in serum testosterone could be related to the alteration of body mass between the experimental groups of bucks.

Table 2: Serum testosterone levels and semen quantitative and qualitative characteristics of mixed-breed goat bucks during the non-breeding season, regarding nutritional status and testosterone treatment. Values are means ± standard deviation

Item	1C	1T	1/2C	1/2T
Testosterone, ng/mL[A]	0.84 ± 1.58[a]	4.78 ± 6.78[b]	0.56 ± 0.96[a]	2.76 ± 4.71[c]
Semen volume (mL)	0.14 ± 0.06	0.33 ± 0.22	0.22 ± 0.19	0.30 ± 0.28
Sperm concentration (x10^6 mL^{-1})	826 ± 276	1037 ± 380	916 ± 389	1026 ± 307
Mass motility (1–5 scale)	1.3 ± 1.0	2.3 ± 1.4	1.6 ± 1.1	1.8 ± 1.5
Sperm progressive motility (%)A	57 ± 11[a]	58 ± 14[a]	52 ± 16[a]	46 ± 15[b]

1C=100% nutrient requirements for maintenance; 1T= 100% nutrient requirements for maintenance plus testosterone; 1/2C= 50% nutrient requirements for maintenance; 1/2T= 50% nutrient requirements for maintenance plus testosterone.
[A]Feeding level x testosterone treatment interaction (P<0.05).
[a,b]Means in the same raw with different superscript differ (P<0.05).

Table 3: Sexual behaviors exhibited by well-fed or underfed goat bucks with or without testosterone administration during the non-breeding season by exposure to estrogenized does. Values are means ± standard deviation

Item	1C	1T	1/2C	1/2T
Anogenital sniffing	10.0 ± 7.6^{a}	59.8 ± 38.1^{b}	11.2 ± 7.2^{a}	26.0 ± 20.1^{a}
Flehmen responses	1.0 ± 0.7	5.2 ± 6.3	0.6 ± 1.3	3.6 ± 4.6
Vocalizations	10.6 ± 12.7^{a}	78.2 ± 30.3^{b}	20.8 ± 33.0^{a}	27.2 ± 18.2^{a}
Approximations to does	12.6 ± 11.3^{a}	82.6 ± 41.5^{c}	33.0 ± 34.5^{ab}	54.0 ± 34.2^{bc}
Mount attempts	0.4 ± 0.5^{a}	12.8 ± 11.6^{b}	1.8 ± 1.3^{a}	7.8 ± 5.6^{b}
Complete mounts	0.0 ± 0.0	0.8 ± 1.3	1.2 ± 1.6	1.8 ± 1.9
Mounts with penis exposed	0.8 ± 1.7^{a}	4.8 ± 3.4^{b}	0.6 ± 0.5^{a}	2.4 ± 1.5^{a}
Mounts with ejaculation	0.4 ± 0.9^{a}	4.0 ± 1.2^{b}	0.6 ± 0.5^{a}	$2.8 \pm 1.^{6}$a
Latency to first mount, sec^{A}	164 ± 43^{a}	82 ± 63^{c}	145 ± 51^{a}	110 ± 44^{b}

1C=100% nutrient requirements for maintenance; 1T= 100% nutrient requirements for maintenance plus testosterone; 1/2C= 50% nutrient requirements for maintenance; 1/2T= 50% nutrient requirements for maintenance plus testosterone.
Values are expressed as total behavioral events recorded (two days; 20 min per day).
^{A}*Feeding level x testosterone treatment interaction (P<0.05).*
^{a,b}*Means in the same raw with different superscript differ (P<0.05).*

Semen volume, sperm concentration and mass motility were not affected by feeding level or exogenous testosterone. This is in contrast to Tufarelli et al. (2011), who reported that semen volume and concentration were positively influenced by concentrate supplementation in rams. Many other studies have found improved semen characteristics when the nutritional condition of rams (Ghorbankhani et al., 2015) and goat bucks (Almeida et al., 2007) was improved. Although Kheradmand et al. (2006) found that neither semen volume nor sperm progressive motility were improved with an enhanced diet in Bakhtiary rams. Many other studies have found improved semen characteristics when the nutritional condition of rams (Ghorbankhani et al., 2015) and goat bucks (Almeida et al., 2007) was improved, although Kheradmand et al. (2006) found no improvement in semen volume with an enhanced diet in Bakhtiary rams. It was expected that semen volume and sperm concentration would be lower in the underfed goat bucks, which had slightly lower scrotal circumference. Kheradmand et al. (2006) argued that in rams, sperm cell concentration was higher after 7 weeks of improved diet administration. However, in the present study, no significant differences among buck groups were recorded for sperm concentrations. An explanation could be found in the fact that this trial only lasted for 29 days, insufficient for the completion of a spermatogenesis cycle in goat bucks of about 7 weeks (Franca et al., 1999). Sperm concentration, in this case, is, therefore, a consequence of the management these animals were subjected prior to the onset of the experiment. Significant differences were observed for sperm cell progressive motility (lowest in the 1/2T group), indicating that the administration of testosterone was not helpful to maintain adequate sperm progressive motility in underfed goat bucks.

In group 1T, goat bucks exhibited more investigatory anogenital sniffing behaviors toward the female stimulus animals compared to the other groups. Likewise, these bucks showed an increased frequency of vocalizations and greater mounts with penis exposed and mounts with ejaculation in response to estrous females. In group 1T, bucks exhibited a notable enhancement of almost all sexual behavior characteristics as it has been demonstrated previously (Ángel-García et al., 2015a,b) following exposure to stimulus females. So, it could be considered that the greater investigatory behaviors exhibited by these goat bucks derived from an enhanced neuroendocrine response due to greater blood levels of testosterone in these animals, in response to testosterone administration.

The ejaculation is the culmination of the recognition and preparatory behaviors in the courting of does, therefore, it is an important indicator of the libido and motivational state of bucks. In the present study, there was a positive relationship between the precopulatory behaviors and the mounts accompanied with ejaculation, which supports the view that the frequency of precopulatory behaviors in goat bucks reflects their underlying sexual motivation.

The precopulatory response toward estrous does by the underfed and saline-treated bucks could be due, at least in part, to their low body energy reserves and above all, to their low serum testosterone levels. This situation apparently limited their investigatory interest toward the estrogenized does. In low-performing and male-oriented rams, the sensory signals exuded by the estrous females are neither detected nor sufficiently provocative to elicit further investigation by the ram (Alexander et al., 1999).

Latency to the first mount, a good index of libido, was much shorter in the group1T compared to the other groups. The adequate feeding level combined with exogenous testosterone had an enhancing effect on time to first mount. As it has been noted by Zarazaga et al. (2009), in goat bucks, when the level of nutrition increased, sexual activity increased, too. Additionally, testosterone concentrations are directly linked to sexual and aggressive behavior in rams (Ungerfeld and Lacuesta, 2015).

The flehmen reaction was expressed more frequently in the testosterone-treated goat bucks. In this species, flehmen response was displayed when a determination of estrus occurred (Ungerfeld et al., 2006), while goat bucks receive information from females through the primary olfactory mode. Thus, well-fed bucks with higher blood testosterone levels were more competent to detect odorants derived from receptive goats.

CONCLUSIONS

This study provides evidence that high levels of exogenous testosterone administered to sexually inactive mature mixed-breed bucks at 25° N provoke a perceptible sexual activity, which suggests that exogenous testosterone in the non-breeding season stimulates cerebral structures mediating male sexual drive. However, this response is mainly attained in well-fed goat bucks. Therefore, supplementary feed should be supplied in conjunction with testosterone to goat bucks during the nonbreeding season in order to potentiate their sex drive.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

Al-Sobayil KA, Zeitoun MM, Khalil MH Abdel-Salam M (2008) Effect of oral administration of a functional symbiotic syrup on libido, semen characteristics, serum testosterone and liver and kidney function of goat's bucks. Asian J Biol Sci 1: 11-18.

Alexander BM, Stellflug JN, Rose JA, Fitzgerald JD, Moss GE (1999) Behavior and endocrine changes in high-performing, low-performing, and male-oriented domestic rams following exposure to rams and ewes in estrus when copulation is precluded. J Anim Sci 77: 1869–1874.

Almeida AM, Schwalbach LMJ, Cardoso LA, Greyling JPC (2007) Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. Small Rumin Res 73: 216–220.

Ángel-García O, Meza-Herrera CA, Contreras-Villarreal V, Guillen-Muñoz JM, Leyva C, Robles-Trillo PA, Rivas-Muñoz R, Rodríguez-Martínez R, Mellado M, Véliz FG (2015a) Effect of different male-to-female ratios and testosterone administration upon the male sexual behavior and the out-of-season reproductive response of anestrous goats. Small Rumin Res 123: 21-29.

Ángel-García O, Meza-Herrera CA, Guillen-Muñoz JM, Carrillo-Castellanos E, Luna-Orozco JR, Mellado M, Véliz-Deras FG (2015b) Seminal characteristics, libido and serum testosterone concentrations in mixed-breed goat bucks receiving testosterone during the non-breeding period. J Appl Anim Res 4: 457-461.

Barkawi AH, Elsayed EH, Ashour G, Shehata E (2006) Seasonal changes in semen characteristics, hormonal profiles and testicular activity in Zaraibi goats. Small Rumin Res 66: 209–213.

Bielli A, Pedrana G, Gastel MT, Castrillejo A, Moraña A, Lundeheim N, Forsberg M Rodriguez-Martinez H (1999) Influence of grazing management on the seasonal changes in testicular morphology in Corriedale rams. Anim Reprod Sci 56: 93–105.

Blache D, Chagas LM, Blackberry MA, Vercoe PE, Martin GM (2000) Review Metabolic factors affecting the reproductive axis in male sheep.J Reprod Fert 120: 1-11.

Detweiler G, Gipson T, Merkel R, Goetsch A, Sahlu T (2008) Body condition scores in goats In: Proceedings 23rd Annual Goat Field Day, Langston University, Langston, OK. pp 127-133.

Fernández M, Girálde FJ, Frutos P, Lavín P, Mantecón AR (2004) Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behavior of mature Assaf rams. Theriogenology 62: 299–310.

Fourie PJ, Schwalbach LM, Neser FWC, Van der Westhuizen C (2004) Scrotal, testicular and semen characteristics of young Dorper rams managed under intensive and extensive conditions. Small Rumin Res 54: 53–59.

Franca LR, Becker-Silva SC, Chiarini-Garcia H (1999) The length of the cycle of seminiferous epithelium in goats (Capra hircus). Tissue Cell 3: 274-80.

Ghorbankhani F, Souri M, Moeini MM, Mirmahmoudi R (2015) Effect of nutritional state on semen characteristics, testicular size and serum testosterone concentration in Sanjabi ram lambs during the natural breeding season. Anim Reprod Sci 153: 22–28.

Hötzel MJ, Walkden-Brown SW, Fisher JS, Martin GB (2003) Determinants of the annual pattern of reproduction in mature male Merino and Suffolk sheep, responses to a nutritional stimulus in the breeding and non-breeding seasons. Reprod Fert Dev 15: 1–9.

Iwata E, Wakabayashi Y, Kakuma Y, Kikusui T, Takeuchi Y, Mori Y (2000) Testosterone-dependent primer pheromone production in the sebaceous gland of male goat. Biol Reprod 62: 806–810.

Karagiannidis A, Varsakeli S, Karatzas G (2000) Characteristics and seasonal variations in the semen of Alpine, Saanen and Damascus goat bucks born and raised in Greece. Theriogenology 53: 1285–1293.

Kheradmand A, Babaei H, Batavani RA (2006) Effect of improved diet on semen quality and scrotal circumference in the ram. Vet Arch 76: 333-341.

Luna-Orozco JR, Guillen-Muñoz JM, De Santiago-Miramontes MA, García JE, Rodríguez-Martínez R, Meza-Herrera CA, Mellado M, Véliz FG (2012) Influence of sexually inactive bucks subjected to long photoperiod or testosterone on the induction of estrus in anovulatory goats. Trop Anim Health Prod 44: 71–75.

Martin GB, Tjondronegoro S, Blackberry MA (1994) Effects of nutrition on testicular size and the concentrations of gonadotrophins, testosterone and inhibin in plasma of mature male sheep. J Reprod Fert 101: 121-128.

Mekasha Y, Tegegne A. Rodriguez-Martinez H (2007) Effect of supplementation with agro-industrial by-products and khat (Catha edulis) leftovers on testicular growth and sperm production in Ogaden bucks. J Vet Med Series A: Physiol Pathol Clin Med 54: 147–155.

Mellado M, Herrera CAM, Arévalo JR, Garcia JE, Véliz FG (2012) Effect of dietary energy intake and somatotropin administration after weaning on growth rate and semen characteristics of Granadina goat bucks. Turk J Vet Anim Sci 36: 338-345.

NRC-National Research Council (2007) Nutrient requirements of ruminants: sheep, goats, cervids, and new world camelids. Washington, DC National Academy Press.

Prathalingam NS, Holt WW, Revell SG, Jones S, Watson PF (2006) The precision and accuracy of six different methods to determine sperm concentration. J Androl 27: 257–262.

Ramadan TA, Taha TA, Samak MA, Hassan A (2009) Effectiveness of exposure to long day followed by melatonin treatment on semen characteristics of Damascus male goats during breeding and non-breeding seasons. Theriogenology 71: 458–468.

Roca J, Martínez E, VázquezJM, CoyP(1992)Characteristics and seasonal variations in the semen of Murciano-Granadina goats in the Mediterranean area. Anim Reprod Sci 29: 255-262.

Tufarelli V, Lacalandra GM, Aiudi G, Binetti F, Laudadio V (2011) Influence of feeding level on live body weight and semen characteristics of Sardinian rams reared under intensive conditions. Trop Anim Health Prod 43: 339–345.

Ungerfeld R, Lacuesta L (2015) Competition between different social ranked rams has similar effects on testosterone and sexual behaviour throughout the year. Reprod Dom Anim 50: 1022–1027.

Ungerfeld R, Ramos MA, Möller R (2006) Role of the vomeronasal organ on ram's courtship and mating behaviour, and on mate choice among oestrous ewes. Appl Anim Behav Sci 99: 248–252.

Van Lancker S, Van Den Broeck W, Simoens P (2005)Morphology of caprine skin glands involved in buck odour production. Vet J 170: 351-358.

Walkden-Brown SW, Restall BJ, Norton BW, Scaramuzzi RJ, Martin GB (1994) Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. J Reprod Fert 102: 351–360.

Zarazaga LA, Guzmán JL, Domínguez C, Pérez MC, Prieto R (2009) Effects of season and feeding level on reproductive activity and semen quality in Payoya buck goats. Theriogenology 71: 1316–1325.