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Relationships between physiological and behavioral responses of goat bucks in mating season

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ABSTRACT - The objective of this study was to determine the sexual parameters in young bucks with different sexual activity and the effect of experience (age) on sexual parameters in bucks. Fifteen young, sexually inexperienced (SI) and five adult, sexually experienced (SE) Saanen × Hair goat crossbred bucks were used in this study. Bucks were individually exposed to three estrus does for 15-min periods during four sexual performance tests, which were carried out every other day to determine some sexual behavior traits such as flehmen response, reaction time, and ejaculation efficiency. Sexually inexperienced young bucks were evaluated based on their sexual activity (active (AC) vs inactive (IA)) and presence of horn. It was found that AC bucks had significantly higher serum testosterone concentrations (31.5 vs 21.5 ng/mL) and weight loss (114.2 vs 51.2 g/day) than IA bucks. In addition, weight loss was significantly correlated with ejaculation efficiency in young bucks. On the other hand, horned bucks tended to have higher ejaculation efficiency with significantly longer reaction time than polled bucks. For experience (age) classes, SE bucks had higher testosterone concentration (76.0 vs 31.5 ng/mL) and weight loss (16.7 vs 8.6%) than SI active young bucks. However, the effect of experience on other sexual performance traits was found to be limited. Overall, these results indicate that testosterone concentration may affect sexual performance of young bucks in the mating season. Age-related sexual experience is not a determining factor for sexual performance in bucks. However, sexual performance data alone may not reflect reproductive outcomes without semen data.

Key Words: copulation, experience, reaction time, test-day, testosterone

Introduction

The sexual behavior of goats is an important factor for flock breeding efficiency and productivity in goat farming. Male fertility is a vital issue because numerous does are generally mated to a single buck. On the other hand, if sexual behaviors are expressed and semen is collected at earlier ages, these could be used in breeding programs to shorten the generation interval. Therefore, evaluation of male fertility using a serving capacity test prior to mating is good practice to reach breeding success.

In goat bucks, courting (latency and amount of courting anogenital sniffing, vocalization, nudging, and flehmen) and copulatory (mounting and ejaculation) behaviors are evaluated using a sexual performance test that is part of a serving capacity test (Imwalle and Katz, 2004; Darwish and Mahboub, 2011).

Serving capacity and sexual performance tests can be used to predict pasture-mating efficiency in rams (Perkins and Roselli, 2007). However, these types of test are not common practices for small ruminants and there is less interest and fewer studies associated with sexual performance in goats compared with other livestock species (Flores et al., 2000; Darwish and Mahboub, 2011; Moghaddam et al., 2012).

Testosterone concentration is known to affect spermatogenesis, the expression of sexual behavior, and secondary sexual traits (Roselli et al., 2002; Perkins and Roselli, 2007). However, the factors affecting the variation in peripheral testosterone concentration are not well understood. Moreover, few studies have been conducted to determine the variation of testosterone concentration and its relationship with sexual performance of young males in mating season (Preston et al., 2012).

A positive relationship between presence of horn and semen characteristics has been reported (Al-Ghalban et al., 2004). Better fertility results were observed when horned bucks were used (Rekik et al., 2012). However, the effect of presence of horn on sexual performance needs clarification.

Sexual performance can vary depending on experience, age, and time of first exposure to estrus female (Perkins and

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Roselli, 2007). Previous studies have indicated that older males had higher ejaculation efficiency (Kridli and Said, 1999; Price et al., 1991) and shorter reaction time (Darwish and Mahboub, 2011) than younger rams and bucks.

The present study was undertaken to determine the sexual parameters (sexual performance test findings, testosterone concentrations, and scrotal circumference) in young bucks with different sexual activity and the effect of experience (age) on sexual parameters in bucks.

Material and Methods

The study was carried out on an experimental farm (38° 34' 1" N 43°17'30" E and 1700 m above sea level) located in the city of Van, in 2014. Van is a city in the eastern region of Turkey with a harsh continental climate of cold, snowy winters and warm, dry summers (Peel et al., 2007). During the period in which the study was conducted, the average air temperature was 17 °C and average monthly precipitation was 14.3 kg/m^2 according to long-term meteorological data (1950-2015) (Anonymous, 2016). The animal material used in the study consisted of 15 sexually inexperienced (SI; with an average age of 1.5 years; first service season) and five sexually experienced (SE; with an average age of 3.8 years; ≥ two service seasons) Saanen × Hair goat crossbred bucks; and 12 Hair goat does (with an average age of 4.1 years). All bucks (five adult + fifteen young) were exposed to 120 does in total. Polled:Horned ratio was 8:7 for inexperienced bucks and 3:2 for adult bucks. Goats grazed on pasture two times a day for eight hours (from 06.00 h to 10.00 h and from 15.00 h to 19.00 h) and for the rest of the day they were penned with no extra feed supplementation. Species existing in the native pasture were 22 species of Poaceae (*Aegilops* spp, *Bromus* spp, and others.), 25 species of Fabaceae (*Astragalus* spp, *Vicia* spp, *Trifolium* spp, *Medicago* spp, *Lotus corniculatus*, and others), 17 Asteraceae, eight Caryophyllaceae, five Cyperaceae, and 16 other species.

The experiment was carried out at the onset of the breeding season (from September 1st to 30th, 2014). During this period of time, all bucks were with the does, except on the days on which sexual performance tests were performed. Four sexual performance tests were carried out in the middle of September (from September 13th to 19th) as described by Price (1993). Visual and olfactory contacts between bucks and does were prevented before the sexual performance tests. Tests were carried out in a 4 × 5 m area by penning bucks individually with three unrestrained estrous does for 15 min. Testing was conducted every other day and carried out between 07.00 h and 13.00 h. The bucks to be tested were selected randomly to eliminate the effect of the day. All bucks were tested for the flehmen response (FR), latency to first mount (FM: the time from entry into the pen to the first mount), number of mounts (nM: attempts to mount or mount without pelvic oscillation), reaction time (RT: the time between the exposure of a male to estrus female and ejaculation), number of ejaculations (nE), and ejaculation efficiency (EE). Ejaculation efficiency was calculated as follows: Ejaculation efficiency = mounts with ejaculations / (mounts with ejaculations + mounts without ejaculations). Sexual behavior was observed by two researchers; one researcher monitored the behavior traits while the other recorded the data.

After sexual performance tests, young bucks were divided into two sexual activity groups as active or inactive. Based on Perkins et al. (1992), bucks which mounted at least ten times and had one ejaculation during the last two tests were classified as active bucks. For experience (age) analysis, the old, sexually experienced (SE) bucks were compared with young, active, sexually inexperienced (SI) bucks.

The presence or absence of horns was recorded for each buck. Only inexperienced bucks were used to determine the effect of presence of horn. The body weights of animals were recorded at the beginning and end of the experiment (from September 1st to 30th, 2014). At the beginning of the study, scrotal circumference (cm) was measured with a flexible tape placed around the scrotum.

Testosterone concentrations (TC) of bucks were determined by using blood samples that were collected twice on the same day (on the same day as body weight measurements) from *vena jugularis* of each buck. On each sampling day, 10 mL blood was collected at 07.00 h and 17.00 h. The blood samples were centrifuged at 3000 × g for 15 min, then serum was harvested and stored at –80 ºC until assayed. Serum testosterone concentrations were assayed by using a goat testosterone commercial ELISA kit (CSB-E13630G, Cusabio Biotech Co. Ltd., Wuhan, China) according to the manufacturer's instructions. The detection range of the kit is 0.1-20.0 ng/mL and sensitivity is 0.05 ng/mL. Coefficient of variation of the kit is below 15%. The samples with higher testosterone concentration than the highest standard were diluted with phosphate-buffered saline.

Data were analyzed using the SPSS 22.0 software to determine the effects of sexual activity, presence of horn, experience (age), and test-day as the main factors affecting sexual behavior traits, body measurements, and testosterone

concentrations. The normality tests of all variables were performed with the Shapiro-Wilk test. The variables that had non-normal distribution such as FR, FM, nM, RT, nE, and EE were analyzed by Mann-Whitney U and Kruskal-Wallis H tests. Testosterone concentrations of bucks were analyzed by the General Linear Model method using the following equation:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + e_{ijkl},$$

in which Y_{ijkl} = the value of the examined characteristic for the *l*-th animal in the *k*-th sampling time and the *j*-th sampling day from *i*-th sexual activity, experience (age), and presence of horn; μ = overall mean; α_i = the fixed effect of sexual activity (active or inactive), experience (age) (experienced or inexperienced), and presence of horn (horned or polled); β_j = fixed effect of sampling day (initial day or final day); τ_k = fixed effect of sampling time (morning or evening); and e_{ijkl} = random error.

Body weights and scrotal circumference of bucks were analyzed with the independent samples t-test. Spearman rank correlation coefficient was used to determine the relationships among traits. In addition to this, multiple correspondence analysis was used to represent projection of the relationship among categorical variables. The categorization of the testosterone concentration and live weight loss were carried out according to frequency distributions of these variables.

Results

After sexual performance tests, it was determined that young bucks displaying low sexual activity accounted for approximately 50% of all young bucks. There were no significant differences between active (AC) and inactive (IA) yearling bucks in terms of body weight and scrotal circumference. On the other hand, TC, live weight loss, and sexual performance traits were significantly different between AC and IA young bucks (P<0.05) (Tables 1 and 2). There were no significant differences between horned and polled bucks in terms of physical traits and serum testosterone concentrations (Table 1).

The findings of serum testosterone analysis showed that AC bucks had significantly higher testosterone concentrations than IA bucks, while testosterone concentrations did not significantly vary according to sampling time (P = 0.419) (Figure 1).

The expressions of sexual behavior throughout sexual performance testing were found to be significantly different between AC and IA bucks. It can be seen that AC bucks had higher FR, nM, nE, and EE than IA ones (Table 2). On the other hand, FM and RT were low in polled bucks, whereas the EE tended to be higher in horned young bucks in the present study.

Table 1 - Body weight changes, scrotal circumference (mean ± SE) and serum testosterone concentrations (Least square mean ± SEM) of young bucks according to sexual activity and presence of horns

Trait	Sexual activity		P-value	Presence of horns		P-value
	Active	Inactive		Polled	Horned	
Number of bucks	8	7		8	7	
Scrotal circumference (cm)	25.7±0.76	25.6±0.64	0.924	26.3±0.79	25.1±0.56	0.243
Initial body weight (kg)	38.3±0.91	37.3±1.36	0.572	37.1±0.94	38.6±1.22	0.342
Final body weight (kg)	35.0±0.89	35.9±1.05	0.527	35.1±0.59	35.7±1.18	0.696
Weight loss (%)	8.6±0.84	3.7±1.74	0.020	5.0±1.73	7.5±1.37	0.266
Daily weight loss (g)	114.2±10.80	51.23±23.82	0.026	66.5±22.60	100.9±18.67	0.257
Serum testosterone (ng/mL)[1]	31.5±3.26	21.5±3.49	0.038	27.7±3.65	26.1±3.41	0.740

SE - standard error; SEM - standard error of the mean.
[1] Average of sampling days.

Table 2 - Sexual performance (mean ± SE) of young bucks according to sexual activity and presence of horns

Trait[1]	Sexual activity		P-value	Presence of horns		P-value
	Active	Inactive		Polled	Horned	
Number of observations	32	28		32	28	
Flehmen response	2.9±0.30	1.7±0.30	0.007	2.0±0.32	2.6±0.30	0.154
Latency to first mount (min)	2.0±0.56	1.9±1.09	0.783	0.6±0.22	2.5±0.67	0.031
Number of mounts	15.5±3.00	5.3±2.32	<0.001	5.0±1.56	15.7±3.33	0.001
Reaction time (min)	3.5±0.75	5.5±1.31	0.095	1.4±0.31	5.0±0.83	0.006
Number of ejaculations	1.0±0.17	0.2±0.09	<0.001	0.6±0.20	0.7±0.11	0.117
Ejaculation efficiency	0.18±0.045	0.03±0.019	<0.001	0.08±0.038	0.14±0.038	0.052

SE - standard error.
[1] Average values of sexual behaviors in four tests.

Highly significant correlations were found between body weight loss and some sexual performance traits (Table 3). Flehmen response , nM, and nE were significantly correlated with weight loss (P<0.001) (Table 3). In other words, as sexual activity of bucks increased the loss in live weight increased. There was a significant correlation between scrotal circumference and testosterone concentration (P<0.05).

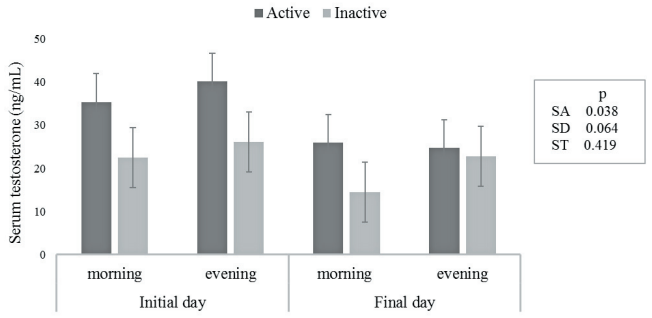
Two dimensions explained 57.1% of the total variation defined by multiple correspondence analysis. The first dimension explained 38.3% of total variation. Sexual activity was correlated with being horned, higher weight loss (%), testosterone concentration, and libido, i.e., AC bucks are located on the right side of the first dimension. The second dimension explained 18.8% of variability and was characterized by Test 1, 3, and serum testosterone concentrations (Figure 2).

It was determined that SE bucks had higher live weight loss than SI bucks during the experiment (Table 4). SE bucks had higher serum testosterone concentrations than SI bucks, indicating that testosterone concentrations were significantly affected by age. However, TC did not differ according to sampling day or time (Figure 3).

The results of the sexual performance tests in the present study showed that experience had no significant effect on sexual behavior traits, except FR (Table 5).

Table 3 - Significant Spearman rank correlation coefficients (r_s) between sexual behavior and scrotal circumference and testosterone concentration in young bucks

Trait	r_s	P-value
Scrotal circumference - testosterone concentration	0.264	0.041
Flehmen response - weight loss	0.481	<0.001
Number of mounts - weight loss	0.496	<0.001
Number of ejaculations - reaction time	–0.655	<0.001
Number of ejaculations - flehmen response	0.395	0.002
Number of ejaculations - weight loss	0.537	<0.001
Number of ejaculations - number of mounts	0.487	<0.001
Ejaculation efficiency - weight loss	0.500	<0.001



SEM - standard error of the mean.

Figure 1 - Serum testosterone concentrations (least square mean ± SEM) determined at different sampling times (ST; morning vs afternoon) of sampling days (SD; initial vs final day) in young bucks having different sexual activity (SA; active vs inactive).

Table 4 - Body weight changes, scrotal circumference (mean ± SE) and serum testosterone concentrations (least square mean ± SEM) in sexually inexperienced (first service season) and experienced (≥2 service seasons) bucks

Trait	Inexperienced bucks[1]	Experienced bucks	P-value
Number of bucks	8	5	
Scrotal circumference (cm)	25.7±0.76	29.3±1.89	<0.001
Initial body weight (kg)	38.3±0.91	70.5±5.04	<0.001
Final body weight (kg)	35.0±0.89	58.8±4.86	<0.001
Weight loss (%)	8.6±0.84	16.77±1.03	<0.001
Testosterone (ng/mL)[2]	31.5±5.86	76.0±7.42	<0.001

SE - standard error.
[1] Only sexually active young bucks were evaluated.
[2] Average of sampling days.

Table 5 - Sexual performance (mean ± SE) of sexually inexperienced (first service season) and experienced (≥2 service seasons) bucks

Trait[1]	Inexperienced bucks[2]	Experienced bucks	P-value
Number of observations	32	20	
Flehmen response	2.9±0.30	0.9±0.19	<0.001
Latency to first mount (min)	2.0±0.56	1.9±0.75	0.992
Number of mounts	15.5±3.00	17.3±2.83	0.301
Reaction time (min)	3.5±0.75	4.3±1.08	0.521
Number of ejaculations	1.0±0.17	1.2±0.19	0.402
Ejaculation efficiency	0.18±0.045	0.10±0.022	0.531

SE - standard error.
[1] Average values of sexual behaviors in four tests.
[2] Only sexually active young bucks were evaluated.

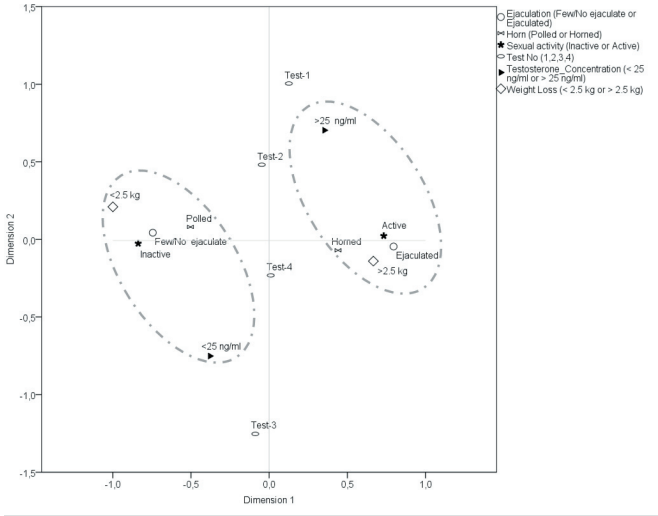
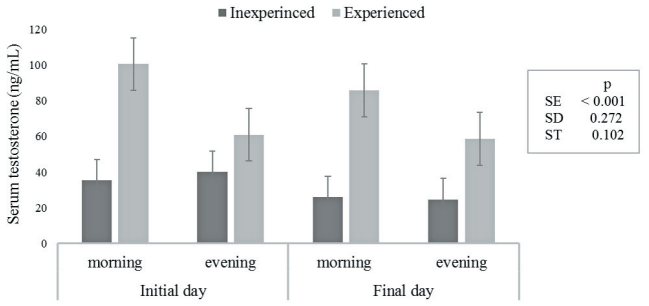


Figure 2 - Multiple correspondence analysis for some physiological and behavioral variables of young bucks.



SEM - standard error of the mean.

Figure 3 - Serum testosterone concentrations (least square mean ± SEM), determined at different sampling times (ST; morning vs afternoon) of sampling days (SD; initial vs final day) in bucks having different sexual experience (SE; experienced vs inexperienced).

Discussion

The live weight loss of bucks depends on various factors such as feed supply and climatic conditions, mating places, buck to doe ratio, and individual variations in sexual activity in the mating season. The differences between live weight loss of AC and IA young bucks observed in the present study might be associated with sexual activity (Table 1). A previous study demonstrated that there was a positive relationship between weight loss and number of servings during the peak of the mating period (Gizaw and Thwaites, 1997).

In general, horned goats were reported to be heavier and more aggressive than polled goats and to dominate them (Miranda-de la Lama and Mattiello, 2010). In addition, fertility improved when using horned rather than polled bucks during the breeding season (Al-Ghalban et al., 2004; Rekik et al., 2012). However, the findings of the present study showed that the presence of horn did not significantly affect body weight, scrotal circumference, and TC (Table 1). It appears that the relationships between being horned and scrotal circumference and TC are not clearly seen in sexual performance traits at younger ages. Since all horned bucks were not sexually active, TC was found to be insignificant.

It is well known that serum testosterone concentrations, semen characteristics, and sexual activity could vary depending on season. It has been reported that testosterone concentrations and sexual performance are significantly high in the mating season (Ahmad and Noakes, 1995; Souri and Mirmahmoudi, 2014). On the other hand, some studies focused only on the variation of sexual performance and its relationship with testosterone concentrations in the mating season (Perkins et al., 1992). Perkins et al. (1992) reported that testosterone concentrations of rams with high sexual performance were higher than rams with low performance in the mating season. In agreement with this study, it was observed that AC bucks had significantly higher serum testosterone levels in comparison with IA bucks (Table 1 and Figure 1) and AC bucks tended to have shorter RT than IA bucks (3.5 vs 5.5 min) (P = 0.095). Previous studies reported that the reduction in RT with increase in testosterone concentrations (Kishk, 2008; Karaca et al., 2015) and elevated testosterone concentrations (Borg et al., 1992) tended to be associated with courtship behaviors.

During the trial, bucks were with does and mating was continuing. So, it appears that copulation activity during the breeding season had a slight effect on testosterone levels of bucks. Therefore, TC tended to decrease on the final day compared with the initial day of the trial (P = 0.064) (Figure 1). Preston et al. (2012) and Dufour et al. (1984) reported that testosterone levels in rams were high at the beginning of the mating season, and then declined through the course of the mating season. However, Illius et al. (1976) reported that the testosterone profiles of young rams were not affected by copulation.

Although Perkins and Roselli (2007) reported that some sexually inactive rams would begin to copulate if provided with additional opportunities, the results of the present study demonstrated that sexual performance tests did not improve the sexual activity of young bucks (P = 0.427) (data are not given). This finding is in line with the finding that bucks do not require sexual experience with estrus females to reach complete serving capacity (Price et al., 1998). However, Imwalle and Katz (2004) suggested that additional opportunities are useful to learn important cues from does to improve sexual experience. Similar findings were indicated by other researchers for rams (Snowder et al., 2002; Stellflug and Berardinelli, 2002). Various factors were reported to affect the findings of sexual performance tests such as sexual motivation, experience, separation anxiety, sexual attractiveness of partner, length of testing period, etc. (Snowder et al., 2002; Imwalle and Katz, 2004). In the present study, it was observed that some of the IA young bucks displayed separation anxiety when removed from pen mates to the test site. In addition, three of the IA young bucks never mounted does. Similar findings reported that 28-40% of yearlings that had no previous sexual experience were sexually inactive during the first test with estrus females (Price et al., 1994; Snowder et al., 2002).

The findings of the present study reveal that the sexual motivation of AC bucks was underlined by higher FR, nM and nE, EE, and TC (Table 2). These results are in line with findings of Perkins et al. (1992). A number of

studies have illustrated that several factors such as breed, climatic conditions, and individual differences affect sexual performance in bucks. In tropical conditions, for example, a 61.98 sec RT was recorded for Saanen bucks, whereas it was 34.25 sec for Nubian bucks (Kamal et al., 2005). Conversely, Darwish and Mahboub (2011) demonstrated that Egyptian-Nubian and Damascus bucks had longer reaction times (1.34 vs 3.82 min, respectively), which are in agreement with the present study. This discrepancy may result from differences in the methods of calculating reaction time, i.e., calculation of the RT during semen collection in restrained animals.

It was reported that testosterone concentration is directly correlated with scrotal circumference (Aller et al., 2012; Mahmoud, 2013; Maksimovic et al., 2016), which is in line with the present study. Ford et al. (2009) demonstrated that there was significant correlation between scrotal circumference and RT in bucks, whereas no significant correlations were present between TC and sexual performance test traits in the present study (Table 3). Previous studies reported that there were significant correlations between testosterone concentration and copulating behavior, such as nM with no ejaculation (r = 0.77) (Mahmoud, 2013), RT (r = –0.54) (Karaca et al., 2015), and courtship behavior, such as naso-genital investigation (Borg et al., 1992).

Mellado et al. (2000) reported that bucks lost 574 g/day under range conditions during the mating season. Also, it was noted that live weight loss was 143 g/day in the first cycle of the mating season (Gizaw and Thwaites, 1997). In present study, the higher percentage of live weight loss in SE bucks compared with SI bucks (388±9.1 g/day vs 110±15.1 g/day) could be attributed to high sexual activity of SE bucks (P<0.001) (Table 3). Although no differences were determined in copulation behaviors between SE and SI bucks during sexual performance tests (Table 5), the weight losses determined in SE bucks could be attributed to mating activities. Preston et al. (2012) reported that the time spent searching for receptive females increases in parallel with age in rams. We observed that during the mating period, SE bucks continually engaged in mating activities, dominating and not allowing younger bucks to mate.

As was expected, SE bucks had significantly higher TC than SI bucks (P<0.001). Similar findings reported that older rams had higher testosterone concentrations than younger ones (Preston et al., 2012; Mahmoud, 2013). Furthermore, the effects of sampling day and time on TC were insignificant (Figure 3).

The findings of the sexual performance test in the present study could suggest that SI bucks were as sexually active as SE bucks. Sexually inactive bucks had higher FR than SE and this might be attributed to lack of experience in identifying the reproductive status of does. However, Darwish and Mahboub (2011) reported that inexperienced bucks had higher latency to anogenital sniffing and RT than experienced bucks. Kridli and Said (1999) reported that yearling lambs exhibited higher nM and leg-kicking bouts than two-year-old rams, while the ejaculation rates were not different between age groups. Similar findings were also reported by some other researchers (Price et al., 1991; Simitzis et al., 2006). By contrast, Santos et al. (2006) observed that young bucks had shorter RT than older bucks in Alpine and Saanen goats.

In summary, findings of this study demonstrated that an important portion of young bucks were sexually inactive and sexual performance tests did not improve the expression of sexual behaviors. Although serum testosterone concentration was not correlated with sexual behavior, there was a significant difference between active and inactive young bucks in terms of average serum testosterone concentration. However, it should be noted that sexual performance alone is not enough for effective fertility without good quality semen. Thus, without having semen data it is hard to establish a reliable relationship between sexual behaviors and hormonal status in bucks.

Conclusions

It can be suggested that serum testosterone concentration affects sexual activity in young bucks during the mating season. Additionally, live weight loss can be related with sexual activity during the mating season. On the other hand, the finding that active young bucks and adult bucks had similar ejaculation efficiency and reaction time could be an indication that age-related sexual experience is not related to sexual performance in bucks.

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