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# Sexual Behavior and Semen Attributes Assessment on Feeding Khejri (*Prosopis cineraria*) Leaves in Malpura Rams of Semi-arid Region

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Khejri (*Prosopis cineraria*) contains substantial amounts of polyphenols like tannin naturally and is preferable fodder by farmers for animal nutrition, having positive attributes for reproductive events like semen quality. However, the effect is not observed in sexual performance and semen attributes in pubertal rams. The present investigation is to study the effects of Khejri leaves feeding on pubertal age, sexual behavior, and semen parameters in weaned ram lambs.

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**Methods:** For the present study, 16 ram lambs were randomly divided into control (C) and treatment (T) considering the body weight and age uniform after weaning. Animals were individually stall-fed, with the Coffered Cenchrus + gram straw (2:1) and T with dried Khejri leaves @ 200 gm at initial two weeks to 1200 kg at advanced age with concentrate mixture common @ 300 gm/day/animal. The lambs were trained for semen donation at the age of 7 months once in a week intervals till attaining puberty. At puberty each male lambs were exposed to a mating session to record the sexual behavior and libido.

**Results:** There was gradual increase in feed intake and body weight in T group rams than C group, resulting in higher mean scrotal morphology and body measurements values. Mounting was higher in T followed by C with non-significant value; however, there was significant ($P<0.05$) difference in duration of ejaculation with lower duration in T. Similarly, the number of ejaculation was also significantly ($P<0.05$) higher in T group.Libido score was similar in C and T group 2.85 and 2.82 respectively. The mean age of puberty was lower ($P=0.16$) in theT group as compared to the C group (232.66±7.96vs 257.4±9.92 days). The seminal parameters like mass motility, sperm concentration and semen volume also found to be significantly higherin tannin fed group than non-fed group.

*Keywords: Ejaculation; libido; polyphenols; sexual behavior; tannin.*

## 1. INTRODUCTION

Sheep rearing in arid and semi-arid regions is challenging due to poor feed and fodder availability due to its agroclimatic conditions and geographical location. In these regions, both grazing and semi-intensive livestock production systems face constraints like low incomes and limited feed resources. It is essential to look for finding alternative and locally available feed resources, crucial for sustaining production year-round. Various nutrient-rich plants act as essential feed sources in these areas. These plants, besides providing nutrition, can also be considered nutraceuticals due to their unique chemical compositions, specifically secondary metabolites with potential biological benefits on animal performance [1]. Among these, polyphenols have drawn substantial interest due to their availability in different plants and beneficial functions. The richness of these plants contributes to antioxidant, anti-inflammatory, metabolism, and immune-modulatory effects along with antihelminthic, antimethanogenic, and antimicrobial properties [2], gaining importance in livestock production [3,4].

Among locally available fodder plants in semi-arid parts, Khejri (*Prosopis cineraria*) naturally contains substantial amounts of polyphenols like tannin and is a preferable fodder increasingly used by farmers for animal nutrition, having positive attributes due to these polyphenols on animal growth, health, performance, and production [5]. Khejri is the most important feed species providing nutrition and good palatability both as green and dry fodder, readily eaten by small ruminants, constituting a major feed requirement of desert livestock. The leaves are of high nutritive value, and phytochemical screening mainly revealed the presence of alkaloids, carbohydrates, proteins/amino acids, glycosides, saponins, flavonoids, and phenolics/tannins in alcohol and water extracts. Approximately 80% of woody perennial dicotyledons and 15% of annual and herbaceous perennial dicotyledon species contain tannins as phenolic secondary compounds [6,7].

Tannins have both adverse and beneficial effects, depending on their concentration and composition in the basal diet [8,9,10]. Dietary tannin helps prevent bloat, enhance protein utilization, and control endoparasites, improving growth, wool, and milk production [11,9,10]. Moreover, dietary tannins may also improve the animal antioxidant status [12,13,14]. However, there is evidence of negative effects on animal homeostasis, especially affecting reproductive events. The presence of secondary compounds, mainly tannins, in a wide range of these plant species constrains their fodder potential. Excess tannins cause toxicity in hydrolysable (HT) form and reduce the nutritive value of some nutrients in the condensed (CT) form. Excess tannins bind to form complexes primarily with dietary proteins, carbohydrates, amino acids, and several minerals, reducing intake and availability, and digestibility of fiber and nitrogen [11,8,9]. They can also drive reproductive waste, such as decreasing ovulation rate and increasing

embryonic loss [15]. However, moderate consumption of tannin has a beneficial effect, improving protein metabolism by increasing amino acid absorption and decreasing urea release [16]. Thus, improving the nutritional condition of the animals exerts a positive effect on their productive and reproductive performance. Feeding with tannin-rich feed seems safer and even positive for reproductive events in both males and females. It is found that the productivity of arid or semiarid local bucks/rams is still relatively low compared to that of bucks/rams originating from subtropical regions, this is because of poor body weight of goats at the age of 1 year [17,18]. This is mainly due to the non-optimal breeding efforts carried out in small ruminants.Tannins, known for antioxidant property, may act as ROS binders/acceptors/scavengers to inhibit the damaging effects of ROS [19]. As seen in males,the use of *Zingiber officinale* (ginger) root extract [20] and *Allium triquetrum* (wild garlic) bulb and leaf extract in rat [21]; *Acacia mearnsii* (Black Wattle) bark [22] and *Vitis vinifera* (Grape) seed tannin extract [23] in sheep restorestestis histopathological alterations, reduces arsenic and lead toxicity, thereby reducing testicular injury, improved testicular and sperm parameters, ameliorating oxidative sperm markers and increase in superoxide dismutase (SOD). In addition, the diverse effects of tannins on the reproductive system aremainly due to their potential metal ion chelation, protein precipitation, and biological antioxidant abilities [19].

Hormones are vital for reproductive events as they signal through various pathways, making their adequate secretion and function essential for reproductive success. Tannin in the form of polyphenols mediates reproductive events through hormone modulation of neurohormones, gonadotropins, or steroidal hormones. It can also regulate spermatogenesis and steroidogenesis, affecting testosterone levels, libido, erectile function, testis development, sperm concentration, and quality [24]. In contrast the hormone release i.e., testosterone is not positively associated with supplementation of tannin in either form [22]. Sexual behavior is critical since it is the initial step for the reproductive cycle. Absence or fade in estrous signs in females or sexual behavior and libido in males lead to reproductive and economic wastage. Polyphenols regulate sexual behavior through different mechanisms, mainly by anti-estrogenic effects that interfere with endogenous estrogen action [25,26]. The effects of polyphenols depend on the concentration of endogenous estradiol (E2) because both polyphenols and E2 compete for binding sites on estrogen receptors (ERs). Polyphenols have an E2 inhibitory role as they act as estrogenic antagonists by occupying part of the ERs. Some polyphenols possess E2 antagonistic action by binding more with ERβ than with ERα [27]. In males, polyphenols have an inhibitory action by binding with ER in the brain, causing changes in reproductive function and performance. They can alter the biosynthesis and function of reproductive hormones along the hypothalamus-hypophysis-gonadal axis and at the gonadal level. Polyphenols can affect steroid synthesis by ovarian granulosa cells or testicular interstitial cells or by altering the sensitivity of these cells to gonadotrophins or the activity of enzymes involved in sex hormone biosynthesis or by inhibiting the activity of steroid 5α-reductase by binding to the enzyme [28]. However, the benefits may be questionable in breeding animals, mainly because improper polyphenol intake may affect the reproductive performance of parents and progeny over a generation due to nutrigenomic and epigenetic changes affecting and regulating gene expression/ programming and thus the future performance and health/disease status of the offspring [29]. Therefore, the present study aims to find the effects of the polyphenolic compound in the form of tannin in Acacia leaves in the semi-arid region on pubertal male ram's sexual behavior, which will help in deciding the male to be utilized as breeding animals in the future.

## 2. MATERIALS AND METHODS

### 2.1 Site of Study

The study was carried out at the experimental animal farm of the Indian Council of Agricultural Research-Central Sheep and Wool Research Institute (ICAR-CSWRI), Avikanagar, located in the semi-arid region of India having altitude 320m above mean sea level, Longitude 75°28´E and Latitude 26°26´N. The annual temperatures range between 3°C and 46°C with annual relative humidity (RH) between 10% and 85%. The rainfall in this area is erratic and distributed throughout the year with annual precipitation from 200 to 500 mm. The mean monthly

maximum and minimum temperatures ranged from 23.5 to 41.2° C and from 9.2 to 31.5°C, respectively. The feeding trial was carried out from May, 2020 till January, 2021. However, the mating behavior was recorded during October to December, 2020. The mean environmental temperatures, RH, and temperature-humidity index (THI) recorded during the study period were 34°C, 55% and 22, respectively which is considered to be comfortable season for sheep in the semi-arid region of country.

## 2.2 Experimental Animals

The present experiment was conducted on sixteen native Malpura male lambs of 3 to 4 months of age. These animals are characterized as medium to large, with white coat and very light brown face upto brisket region. Malpura sheep derived its name from small town Malpura, of Tonk districts, Rajasthan (India). This sheep is widely distributed breed and predominates in Jaipur, Tonk and Swai Madhopur districts, however it is also marginally distributed in Ajmer, Chittorgarh and Bhilwara districts of Rajasthan, India [30]. This breed is characterized for their hardiness and adaptability to the local climatic condition of semi-arid region.

## 2.3 Animal Management

The winter season lambing is considered as major season lambing for Indian sheep (Mehta et al., 2003). The lambs of this season for 3 months allowed to suckle their mother. After 1 month of age, lambs were offered with a creep feed mixture and adlib hay. Creep feed mixture include barely 650 g/kg, groundnut cake 320 g/kg, minerals 30 g/kg including 10 g/kg NaCl (Crude protein 180 g/ kg and total digestible nutrients 650 g/kg).

## 2.4 Experimental Procedure

After 3 months of age lambs were weaned and male lambs were selected to form two groups, control (C) and treatment (T) group randomly considering the body weight and age of the two groups' uniform. All animals were kept individually in separate feeding unit inside the same experimental shed separated by a chain-linked wire partition, provided with feeding trough and water trough. The C group were offered Cenchrus + Chana Bhusa (2:1) and T group with dried Khejri leaves (15% tannin on DM basis) @ 200 gm at initial two weeksto 1200 kg with advancement of age with concentrate mixture common @ 300 gm/day/animal. The feed was offered in the morning 09:00 h and leftover feed was collected next morning to calculate the feed intake for the individual animal. Body weight, body measurement and scrotal measurement was taken fortnightly. After continuous feeding for 5 to 6 months animals were prepared for semen donation to find the pubertal age and study of seminal characteristics. The lambs were subjected to training for semen donation by artificial vagina method at the age of 7 months and semen collection was attempted at once in a week interval till attaining puberty in terms of ejaculation with at least 50% motile spermatozoa or lambs reached to age of 10 months, whichever is earlier. The semen sample was evaluated for volume, mass motility and concentration just after collection. The animals when started to donate semen all the animals were utilized for behavioral study at every 15 days. Each male was observed 3 times minimum for recording sexual behavior. Mating behavior recorded after introduction of female to observation pen (5 X 5 sq m).

## 2.5 Data Collection

Recording of mating behavior done on introduction of natural estrus ewe. An observation was made by single observer to avoid an error. Ongoing activities were recorded every 10seconds for 20 minutes. The mating behavior *viz.* vocalization, leg kicking, leg kicking with vocalization, flehmen reaction, sniffing, false mounting (without thrust), mounting and ejaculation was recorded. After behavioral recording reaction time, refractory period and total number of ejaculations was calculated for each ram.

Further, libido and mounting enthusiasm was assessed as per Osborne et al. [31] and Ford et al. [32], respectively.

## 2.6 Data Analysis

The information collected by data sheet was pooled and analyzed as per standard statistical procedure [33]. Data were analyzed by GLM (SPSS 16.0, Chicago, IL, USA). The linear model was used for all the respondent variables using a least-squares analysis of variance and libido score was analyzed using Kruskal Wallis test. The level of statistical significance was set at $p<0.05$. All the data in the experiment were presented as mean ± SE.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect on Feed Intake and Body Weight

After continuous feeding of Cenchrus + Chana Bhusa (2:1) to C group and dried Khejri leaves to T group for 5-6 months, the average feed intake was observed to be lower in C group (403.20±5.30 gm) when compared to T group (479.02±5.34 gm) rams. The weekly feed intake of both group is depicted in Fig. 1. The data reveals that the initial one-month feed intake was low which rose continuously with increment of age in both groupsi.e. 67.66 gm to 708.50 gm in C and 73.81 gm to 760.10 gm in T group with significant (P<0.05) difference.

Mean body weight of pubertal rams for both group is presented in Fig. 2. There was exponential rise in body weight in both the group with advancement of age. However,the body weight gain was superior in rams in T group as compared to C group i.e., 22.05±1.14 kg and 20.69±1.23 kg, respectively, with no significant difference. Both the feed intake and body weight gain are positively correlated in two groups. Feed intake reported to be higher in T group suggesting the palatability of feed, which resulted in added body weight gain in pubertal rams of group T showing no deleterious effect on weight gain.

### 3.2 Effect on Scrotal Biometry

The scrotal biometry of the pubertal rams in different group have been represented in Table 1. The mean testicular length, width, circumferences, and volume found to be significantly (P<0.05) higher in T group when compared with C group. The higher value is directly related to higher feed intake and higher body weight in T group than C group which ultimately affects the testicular biometry of pubertal rams. The relationship between body weight and testicular size is well established [34,35]. The effect of age and body condition score were significant (P < 0.01) on all the testicular measurements in Red Sokoto bucks [36].

### 3.3 Effect on Body Measurements

The body measurements of pubertal rams in different groups have been represented in Table 2. The mean BL, HAW and CG in C and T found non-significant (P > 0.05) difference from each other; however, the value was higher in T group but only PG in T group observed to have significant higher value than C group. The values are also indicative of better feed intake and growth performance in T group pubertal rams than C group rams, resulting in higher body measurements parameters.

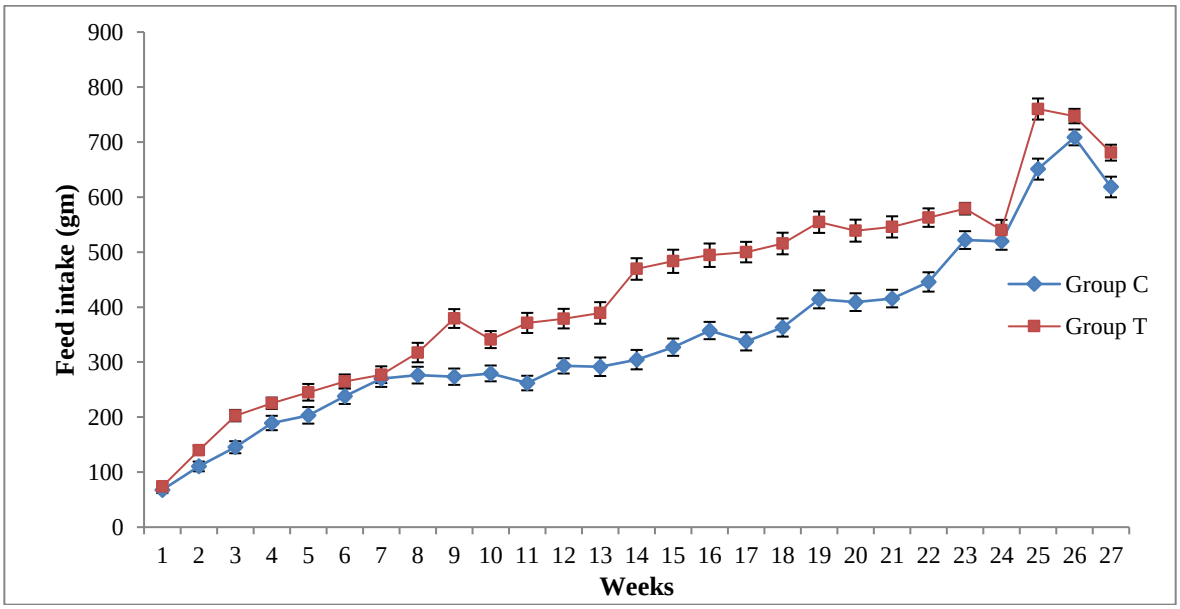


**Fig. 1. Average feed intake (gm) by pubertal rams in two groups (C & T)**

**Table 1. Scrotal morphology (Mean ± SE) of pubertal rams in control and treatment group**

| Parameters | | Control | | | Treatment | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Initial | Final | Mean | Initial | Final |
| Testis length (cm) | R | 5.04±0.21[a] | 3.33±0.62 | 5.53±1.02 | 5.58±0.18[b] | 4.09±0.48 | 6.8±0.37 |
| | L | 5.15±0.20[a] | 3.36±0.61 | 5.57±1.02 | 5.60±0.18[b] | 3.59±0.56 | 6.88±0.31 |
| Testis width (cm) | R | 2.19±0.09[a] | 1.41±0.30 | 2.48±0.52 | 2.61±0.08[b] | 1.94±0.22 | 3.01±0.17 |
| | L | 2.21±0.09[a] | 1.54±0.31 | 2.43±0.51 | 2.63±0.08[b] | 1.96±0.25 | 3.1±0.19 |
| Testis circumference (cm) | | 28.92±0.77[a] | 25.74±2.44 | 28.84±2.57 | 31.84±0.68[b] | 26.70±1.95 | 37.51±1.12 |
| Testis volume (ml) | | 144.49±7.76[a] | 62.69±13.04 | 196.74±34.48 | 199.50±9.47[b] | 94.83±18.11 | 278.35±18.07 |

*Means with different superscript with in a row vary significantly ($p<0.05$)*

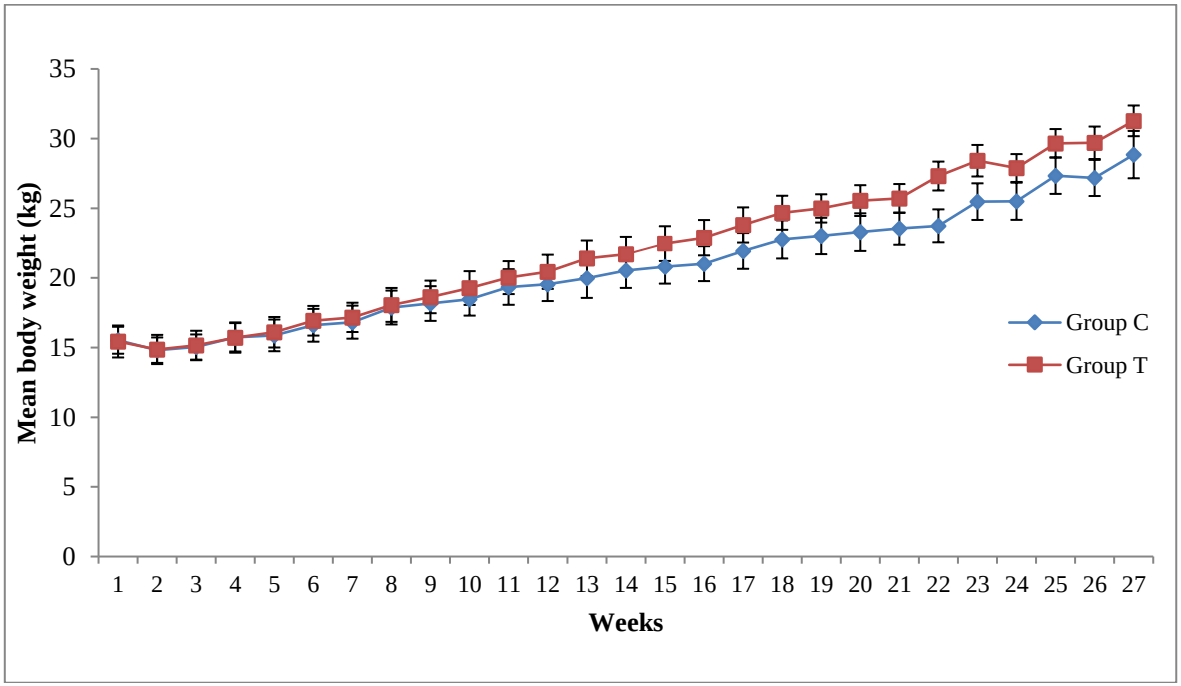


**Fig. 2. Mean ± SE body weight (kg) of pubertal rams in two groups (C & T)**

**Table 2. Body measurements (Mean ± SE) of pubertal rams in control and treatment group**

| | Control | | | Treatment | | |
|---|---|---|---|---|---|---|
| | **Mean** | **Initial** | **Final** | **Mean** | **Initial** | **Final** |
| BL | 61.98±1.51 | 62.13±1.32 | 64.63±1.28 | 63.15±1.49 | 63.75±1.53 | 65.25±1.28 |
| HAW | 64.96±1.66 | 59.75±1.41 | 68.75±1.51 | 66.63±1.91 | 56.75±0.59 | 70.63±0.84 |
| CG | 70.79±1.91 | 71.75±1.33 | 75.38±1.51 | 72.81±1.79 | 74.5±1.82 | 76.88±1.49 |
| PG | 71.9±2.32[a] | 71.38±2.28 | 78.00±1.54 | 75.96±2.31[b] | 76.37±2.10 | 83.00±1.28 |

*Means withdifferentsuperscript with in a row vary significantly (p<0.05)*

## 3.4 Effect on Sexual Behavior

Normative sexual behavior is one of the eight social behaviors reported in sheep [37], is a consequence of the interaction of various factors like physiological, neural, genetic, nutritional, climatic and age. Nutritional factor is important as this helps in regulating the body weight of animals which ultimately responsible for active sexual behavior. Values (Mean ± S.E) of sexual activities in rams of C and T group exposed to single oestrus ewe for 20 minutes mating session are presented in Table 3. For the total involvement of female ram shows courtship behavior before mating and ejaculation. This procedure starts with the ram approaching the ewe and if she stands still and not moving forward, the ram will sniff her perineum. With the start of sniffing ram follows vocalisation and kicking and nudging activity. While urination by ewe or smelling of perineum ram exhibits flehmen reaction and intention mounts, the frequency and duration of which seems to vary widely between rams [38]. The time spent in sniffing, licking, vocalization and nudging was higher in ram lambs of T group than C group but the difference was non-significant. Irrespective of group, sniffing, licking and vocalisation were predominant courtship activities observed. The predominant courtship activities in both group was sniffing and vocalization followed by leg kicking and flehmen reaction, however the values were higher in T group. The higher courtship activities are due to in experience rams and were for first time exposed to estrus ewes. The courtship activities were higher when a single ram was exposed to ewe(s) i.e. either single or multiple ewes for one hour mating session. Among all courtship activities vocalization or leg kicking with vocalization was found to be the predominant teasing activity [39].

But leg kicking and false mount was higher in C group as compared to T group however the result was not significant. False mount was higher in C group, as suggested by comparing ram ages, mature rams spent more time sniffing than the young males [40]. Indicating that the feed rich in tannin is helpful in higher intake resulting in higher body weight gain so the puberty attained is also earlier in other group when compared to control. But here due to non-experience still with high libido pubertal rams of T group show various courtship behavior including nudging. In the present study T group animals exhibit more nudging activity, to show their interest on female and arousing interest on them to mate. It is stated, high rates of frequency of nudging are associated with low sexual efficiency [41] and they are also expressed in the case of non-estrus ewes [42].

Vocalisation with leg kicking and flehmen reaction was almost same in both groups. Mounting was higher in T followed by C with non-significant value however there was significant (P<0.05) difference in duration of ejaculation between two groups with lower duration in T than C group. Similarly, the number of ejaculations was also significantly (P<0.05) higher in T group.Whereas mounting, number of ejaculation and time during ejaculation for T is higher due to attainment of earlier puberty as the feed intake, body weight gain and scrotal measurements were higher than the control. Indicating that the mating behavior is not affected by tannin rich feed indeed it helps in enhancing it by improving feed intake and body weight to improve libido. In contrary in mature male sheep and goats, feed intake contributes a little effect on gonadal endocrine function but have profound effect on sperm production due to changes in size of the seminiferous tubules and in spermatogenic efficiency [43].

Total time spent in standing with interest in ewe was significantly (P<0.05) higher in T group than C group. In contrast the total time spent in standing idle without any mating activities was highest in C group as compared to T group with significant (P<0.05) difference.

**Table 3. Mean ± SE time (min.) spent in different mating activities in pubertal rams**

| Parameters | Control | Treatment |
|---|---|---|
| Sniffing | 55±6.97 | 65.8±10.74 |
| Licking | 41.77±6.63 | 43.42±6.88 |
| Vocalisation | 89.92±9.65 | 90.37±12.87 |
| Leg kicking | 45.72±4.5 | 42.40±7.13 |
| Vocalisation with leg kicking | 22.17±5.02 | 19.87±3.29 |
| False mount | 7.95±2.03 | 5.45±1.67 |
| Flehmen reaction | 9.95±2.9 | 10.22±2.5 |
| Nudging | 19.82±4.8 | 38.50±10.63 |
| Mounting | 8.72±1.34 | 11.52±1.14 |
| Ejaculation | $0.35±0.15^{a}$ (4) | $1.37±0.21^{b}$ (6) |
| No. of ejaculation | $4.3±1.43^{a}$ (4) | $7.83±1.76^{b}$ (6) |
| Standing with interest in female (Seconds) | $159.52±20.75^{a}$ | $335.42±30.90^{b}$ |
| Standing idle (Seconds) | $739.62±36.02^{a}$ | $555.52±48.45^{b}$ |

*Figures in parentheses indicate number of rams that showed activity.*
*Means withdifferent superscript with in a row vary significantly (p<0.05)*

**Table 4. Mean±S.E (min) Introductory Ejaculatory Latency (IEL) and post ejaculatory intervals (PEI) of pubertal lambs**

| | Mean Introductory ejaculation latency (Minutes) | 1st Post ejaculation Interval (Minutes) | 2nd Post ejaculation Interval (Minutes) | 3rd Post ejaculation Interval (Minutes) |
|---|---|---|---|---|
| Control | 1.84±25.58 | $3.11±15.21^{a}$ | $2.88±17.00^{a}$ | $5.36±36.96^{a}$ |
| Treatment | 1.51±18.72 | $2.65±16.60^{b}$ | $2.36±18.40^{b}$ | $6.57±28.34^{b}$ |

*Means with different superscript with in a row vary significantly (p<0.05)*

Mean Introductory ejaculatory latency (IEL) and post ejaculatory interval (PEI) has been depicted in Table 4. IEL for T group male lambs were lower than C group male lambs but the difference was not significant. 1st and 2nd PEI found to be significantly lower (P<0.05) in T group when compared with C group. But the 3rd PEI observed to be significantly (P<0.05) lower in C as compared to T group. The IEL found to be earlier in T which could be due to different level of plasma testosterone hormone affected by different feed. The result was comparable to rams IEL when exposed to single estrus ewe. Exposing male to oestrus female showed IEL of about 28 secondsin comparison to non oestrus females [44], and 6.91 seconds in pubertal bucks [45]. The variation in mean ejaculation latency depends upon the factors like breed, age, season and nutrition. Further, Zarazaga et al. [46] concluded that ejaculation latency was positively influenced by level of feeding. Adequately fed rams reported to have higher (30%) total volume of Leydig cells per testis than in underfed rams, indicating a change in the volume of individual leydig cells [47], resulting in the rate of production of testosterone [48]. In contrast it is also proved that except for extreme under nutrition, these effects are not linked to major changes in testosterone production or in sexual behavior [43]. Similar trend was observed for 1st and 2nd post ejaculation interval (PEI). In 3rd PEI the duration was lower for C group rams as compared to T group, which may be due to initial frequent ejaculation. In fact, during mating, motor activity is greater in rams than in ewes, because of the very active part taken by the rams during courtship [49]. Surprisingly, it is also reported that it is greater in rams with smaller testes than in rams with larger testes [50]. Irrespective of group the time increased with advancement of mating, similar to Shearer andKatz [44] and Kerketta et al. [45] who also reported that PEI increased with advancement of mating.

Initial false mount latency (IFML), Initial mount latency (IML) and Refractory period has been presented in Table 5. IFML was lower in T group when compared to C male lambs, however the difference was non-significant. In contrast IML was lower in C group than T group. In T group rams 1st and 2nd refractory period was significantly (P<0.05) lower as compared to C ram lambs. In both group the increment in the

refractory period in a consistent manner the advancement of mating, except 4th refractory period, here the value observed to be lower and was non significantly higher in C group. IFML was lower in T group when compared to C group male lambs, however the difference was non-significant. This upto an extent indicates that the males in group are interested in female and also due to lack of experience showed this as extended behavior. In contrast IML was lower in C group than T group. The reason may be due to long exposure of C rams standing outside viewing the mating session of T group and directly went for mating with very little pre-courtship activities. In T group rams 1st and 2nd refractory period was lower as compared to C ram lambs. Average values of reaction time in Black Bengal buckswere60.53± 1.223 seconds, in Sannen buck 64.56± 1.233 seconds [51] which was higher than this study. The probable reason for such trend may be due to effect of feed on the hormonal regulation that mainly controls the sexual behavior. In both, refractory period increases with the advancement of mating upto 3rd mating, which is obvious in mating session behavior.

Libido is a sexual desire to mate which refers to sexual motivation, indulge behaviors such as seeking and detecting mate, courtship, and mating [52]. Libido is chiefly measured by reaction time, defined as the elapsed time between exposure to stimuli and first service [53,54]. Libido in males in measured by observing and recording various parameters like based on reaction time, number of ejaculations. Libido was average for pubertal rams in both groups, indicating no deleterious effect of tannin on the sexual desire and performance. Indicating that the plants rich in tannin can be incorporated in feed upto certain extent with no negative impact on sexual behavior of lambs. Overall, it appears that libido is more sensitive to under nutrition than sperm production [43]. Libido for both group was calculated using libido score system. The libido score for C and T group was 2.85 and 2.82 respectively. Libido score was similar in C and T group. In control group one animal showed the score below 1, similar result also found for treatment group. Rams of both groups were mounting with enthusiasm of value +1. Young rams usually show low libido on introduction to a new group [55]. Further it was reported that rams having lack of exposure to ewes during their early life exhibited poor sexual behavior [56].

The mean age of puberty and various seminal parameters is presented in Table 6. It was observed that only 37.5% lambs in T group attained the puberty during the study period as compared to 62.5% in C group. However, the mean age at which lambs attained puberty was lower (P=0.16) in T group as compared to C group (232.66±7.96 vs 257.4±9.92 days).The semen volume, mass motility and sperm concentration were 0.61±0.48 mL, 4.33±0.37 and 3547±414.1 million/mL respectively in the treatment pubertal rams which was comparable and higher than the control group i.e., 0.4±0.08 mL, 2.85±0.52 and 2520±402.0 million/mL respectively. Seminal characteristics may vary according to breed, age, season, and nutrition. So here the effect of nutrition is visible indicating the effect of tannin on seminal parameters. Similarly, semen volume was higher in rams (12-month-old) when supplemented with 1.5 g Tannin extract (TE) and 3g Encapsulated Tannin extract (ETE) than group supplemented with 3g TE and sperm concentration higher when compared to group supplemented with 1.5 g ETE [22]. Condensed tannin as an antioxidant tool splits the negative effect of oxidative chain reaction and improve the process of spermatogenesis, thereby enhance semen quality and general testicle health [57,58]. Moreover, the antioxidant property increases spermatogenesis the positive effect on semen volume and concentration in the present study can be attributed to the potential antioxidant activity in tannin. Very few ram lambs (37.5%) attained pubertal age in the treatment group than control ram lambs (62.5%). Although no significant difference could be drawn but remarkable increase in volume, concentration, and mass was observed. This increase in seminal parameters could be due to higher body weight attained in tannin-fed lambs leading to improvement in body and testicular measurement. The tannin-rich feed may incur the maximum benefit on sperm concentration and motility [22] with a slightly toxic effect on sperm cells. However, the mechanism of positive effect on sperm motility by tannin supplementation is still under investigation [59]. The most important point to consider is the dosage optimization, under and over dose may lead to no or deleterious effect on male reproductive function. As reported the addition of crude guava

**Table 5. Mean±S.E (min) Initial false mount latency, initial mount latency and refractory period of pubertal rams**

| | Initial false mount latency | Initial mount latency | 1st Refractory period | 2nd Refractory period | 3rd Refractory period | 4th Refractory period |
|---|---|---|---|---|---|---|
| Control | 1.06±17.12 | 1.09±18.41 | 0.97±7.26[a] | 1.03±6.05[a] | 1.39±6.05 | 1.04±10.85 |
| Treatment | 0.92±16.51 | 1.15±25.34 | 0.80±6.76[b] | 0.84±5.20[b] | 1.60±13.04 | 0.09±11.64 |

*Means with different superscript with in a row vary significantly (p<0.05)*

**Table 6. Mean±S.E Age of puberty (days) and various seminal attributes of pubertal rams**

| Parameter | Control | Treatment | P-Value |
|---|---|---|---|
| Lambs attaining puberty (%) | 62.5 | 37.5 | 0.61 |
| Age of puberty (Days) | 257.4±9.92 | 232.66±7.96 | 0.16 |
| Semen Volume (mL) | 0.4±0.08[a] | 0.61±0.48[b] | 0.04 |
| Mass Motility (on scale of 0-5) | 2.85±0.52 | 4.33±0.37 | 0.03 |
| Sperm Concentration (Million/mL) | 2520±402.0 | 3547±414.1 | 0.09 |

*Means with different superscript with in a row vary significantly (p<0.05)*

leaf tannins at a concentration of 3% can increase motility, viability, and preserve intact plasma membrane in crossbred goat, however the value above this proved to be toxic destroying the integrity of the spermatozoa plasma membrane that lead to the death of spermatozoa [18]. Kabiraj et al. [60] observed that higher testicular size has higher spermatogenic activity in the bucks of the older age group. Further, it was reported that sperm concentration might vary according to variation in age, breed, collection frequency, feeding regime, and climatic condition [61].

## 4. CONCLUSION

From the present study, it can be concluded that feed definitely controls the reproductive behavior in males. The tannin-rich feed indirectly regulates the body condition and body weight in pubertal rams to enhance puberty, sexual behavior libido, and semen parameters. Feed rich in tannin helps to promote the various courtship activities along with improved mounting and ejaculation than non-tannin feed-fed pubertal rams. Libido was also average for pubertal rams in the group fed with tannin, indicating no deleterious effect of tannin on sexual desire and performance. Similarly seminal quality was also superior with no harmful effect on its physical characteristics. Thus, indicative of plants rich in phytochemicals like tannin can be incorporated in the feed up to a certain extent with no negative impact on sexual behavior of lambs. Further, the nutrigenomics investigation is required to pin point the exact mechanism behind the study.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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