Sexual ~~Behavior~~behavior and semen attributes assessment on feeding Khejri (*Prosopis cineraria*) Leaves in Malpura rams of semi-arid region

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Abstract

Background: Khejri (*Prosopis cineraria*) contains~~found to naturally contain substantial amounts of polyphenols like tannin~~ substantial amounts of polyphenols like tannin naturally and ~~are~~ ispreferable fodder by farmers for animal nutrition, having positive attributes for reproductive events like semen quality. ~~However~~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.

Methods: For ~~present study 16 ram lambs after weaning were randomly divided into two groups: control (C) and treatment (T) considering the body weight and age uniform~~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~~stall-fed, with the C offered Cenchrus + gram straw (2:1) and T with dried Khejri leaves @ 200 gm at initial two weeks to 1200 kg at ~~advance~~ advancedage 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 ~~interval~~ intervalstill attaining puberty. At puberty, each male lambs were exposed ~~for~~ toa mating session to record the sexual ~~behaviour~~behavior and libido.

Result: 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 measurementsvalues. 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 ($P=0.16$) of T group as compared to 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 significantly superior in tannin fed group than non fed group.

~~**Keywords:** Ejaculation~~**Keywords:** Ejaculation;Libido;Polyphenols; Sexual behavio~~u~~r; Tannin

Introduction

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Sheep rearing is a challenging task ~~at~~ inthe region of arid and semi-arid parts of the country due to the poor availability of feed and fodder due to its agroclimatic conditions and geographical location. In semi-arid regions in grazing as well as in ~~semi intensive~~semi-intensive livestock production ~~system~~systems, low incomes and the limitation of feed resources are major constraints, it is essential to look for alternative and available feed resource to sustain the production round the year. There are various plants which are rich in nutrients act as only source of feed in these areas. Moreover, these plants other than acting as food for animals here can also be considered nutraceuticals due to their unique chemical composition, specifically secondary metabolites with potential biological benefits on animal performance.Among the secondary metabolites of plants, polyphenols have drawn substantial interest due to their availability in different plants and desirable beneficial functions. ~~These richness of plants~~This richness of plants contributed in antioxidant, anti-inflammatory, metabolism and immune modulatory effects along with activities of antihelminthic, antimethanogenic and antimicrobial property (Huang*et al.*, 2018) and gaining special importance in livestock production (Mergeduš*et al.*, 2020;Basini*et al.*, 2005).

Among locally available feed fodder plants in semi-arid parts, Khejri(*Prosopis cineraria*) found to naturally contain substantial amounts of polyphenols like tannin and are preferable fodder more and more used by farmers for animal nutrition, having positive attributes in mind of these polyphenols on animal growth, health,performance, and production. 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, 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 (Mueller-Harvey, 1999).

Tannins are considered to have both adverse and beneficial effects, depending on its concentration and composition in basal diet (Makkar*et al.*, 2007;Waghorn, 2008; Piluzza*et al.*, 2014). Dietary tanninis helpful in preventing bloat, enhance protein utilization , endoparasite control, thereby induce improvements in growth, wool and milk production (Min *et al.*, 2003; Waghorn, 2008; Piluzza*et al.*, 2014).Moreover, dietary tannins may also improve the animal antioxidant status (Gladine*et al.*, 2007; López-Andrés *et al.*, 2013).

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However, there is also evidence about the negative effects for animal homeostasis, especiallyaffecting reproductive events. The presence of secondary compounds mainly tannins in a wide range of these plant species constrains their fodder potential. It is proved that tannins in excess causes toxicity in hydrolysable (HT) form, and also responsible for reducing nutritive value of some of nutrients when they are present in the condensed (CT) form. When in excess tannins binds to form complexes primarily with dietary proteins, and also with carbohydrates, amino acids and several minerals, thereby reducing intake and availability, reduction in digestibility of fibre and nitrogen (Min *et al.*, 2003;Makkar*et al.*, 2007;Waghorn, 2008).It can also drive reproductive waste, such as decreasing ovulation rate and increasing embryonic loss (Blache*et al.*, 2008).

However, moderate consumption of tannin rather have a beneficial effect, can improve protein metabolism by increasing amino acids absorption and decreasing urea release (Mueller-Harvey *et al.*,2019). Thus achieving improvement of the nutritional condition of the animals exerting a positive effect on their productive and reproductive performance. Feeding with tannins rich feed seems to be safer and even positive for reproductive events like in Quercushartwissiana (Kaya *et al.*, 2006); Sesbaniasesban (Mekoya*et al.*, 2005); Acacia saligna (Sallam*et al.*,2017); Punicagranatum seed (Zarepourfard*et al.*, 2019).As reported ewes grazing L. corniculatus (tanniferous pasture) had higher ovulation and lambing rates than those grazing perennial ryegrass/white clover pasture (phytoestrogenic pasture) (Sallam*et al.*, 2017).

Hormones are very vital for reproductive events as it signals the event through various pathways and therefore adequate secretion and function is essential for the success of reproductive process. Tannin in form of polyphenols mediate the reproductive events through hormone modulation of either neuro hormones, gonadotropins or steroidal hormones. It can also regulate function of both spermatogenesis and steroidogenesis, as a consequence bring changes in testosterone levels, libido, erectile function, testis development, sperm concentration and quality (Hashem*et al.*, 2020).Among reproductive events sexual behavior is a critical, since it is the initial step for the later reproductive cycle absence or fade in estrous signs in the females or sexual behavior and libido in the males lead to complete reproductive and economic wastage.Polyphenols considered to regulate the sexual behavior through different mechanisms, but mainly by anti estrogenic effects that can interfere with endogenous estrogen action (Hashem*et al.*, 2013; Melaku*et al.*, 2004). In all events, the effects of polyphenols depend mainly on the concentration of endogenous estradiol (E2) because both the polyphenols and E2 compete for the binding sites on estrogen receptor

(ERs). But here polyphenols have E2 inhibitory role as it act as estrogenic antagonists, by occupying a part of the ERs. Further some of polyphenols possess E2 antagonistic action by binding greater with ERβ than for ERα (Lorand*et al.*, 2010). In males polyphenols have inhibitory action by binding with ER in the brain causes changes in reproductive function and performance. These also ~~have ability to~~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 either ovarian granulose ~~cellsor~~ cells ortesticular interstitial cells or by altering the sensitivity of these cells to the action ~~ofgonadotrophinsof~~ gonadotrophins or the activity of enzymes involved in sex hormone biosynthesis or by inhibiting the ~~activityof~~ activity ofthe steroid 5α-reductase by binding to the enzyme (Liu *et al.*, 2008).

~~However~~However, the benefits may be more questionable inbreeding animals; mainly because improper polyphenols intake may not only affect the ~~reproductiveperformance~~ reproductive performanceof parents and progeny over a generation due to nutrigenomic and epigenetic ~~changesaffecting~~changes affecting and regulating gene expression/programming and thus the future performance and health/disease status of the offspring (Amir *et al.*, 2019). Therefore, the present study aims to find the effects of the polyphenolic compound in the form of tannin in Acacia leaves in ~~semi-arid~~semi-arid region on pubertal male ram's sexual ~~behaviour~~ behaviorwhich will help in deciding the male in future to be ~~utilised~~utilized as breeding animals.

MATERIALS AND METHODS

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 ranges between 3° C and 46° Cwith annual relative humidity (RH) between10% 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~~However, the mating behaviour was recorded during October to December, 2020. The mean environmental temperatures, RH, and temperature-

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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.

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 in size, 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 SwaiMadhopur districts, however it is also marginally distributed in Ajmer, Chittorgarh and Bhilwara districts of Rajasthan, India (Kumar *et al.*, 2008). This breed arecharacterized for their hardiness and adaptability to the local climatic condition of semi-arid region.

Animal management, Experimental procedure and data collection

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1. ***Animal management***
2. Experimental procedure
3. Data collection
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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 mixtureinclude 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). 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 insidethe 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 weeks to 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 behavioural study at every 15 days. Each male was observed 3 times minimum for recording sexual behaviour. Mating behavior recorded after introduction of female to observation pen (5 X 5 sq m). Recording of mating behaviour recorded on introduction of natural estrus ewe.An observation was made by single observer to avoidan 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 behaviouralrecording reaction time, refractory period and total number of ejaculation wascalculated for each ram.

Further, libido and mounting enthusiasm was assessed as per Osborne *et al.* (1971) and Ford *et al.* (2009), respectively.

Data analysis

The information collected by data sheet was pooledand analyzed as per standard statistical procedure(Snedecor and Cochran, 1989). Data were analyzed by GLM (SPSS 16.0, Chicago, IL, USA). The linear model was used for allthe respondent variables using a least-squares analysis of variance and libido score was analyzed using Kruskal Wallis test.The level of statisticalsignificance was set at $p<0.05$. All the data in the experiment were presented as mean ± SE.

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Resultsand Discussion

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 Figure 1. The data reveals that the initial one month feed intake was low which rose continuously with increment of age in both group*i.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 figure 2. There was exponential rise in body weight in both the group with advancement of age. Howeverthe 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 is positively correlated in two groups. Feed intake reported to be higher in T

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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.

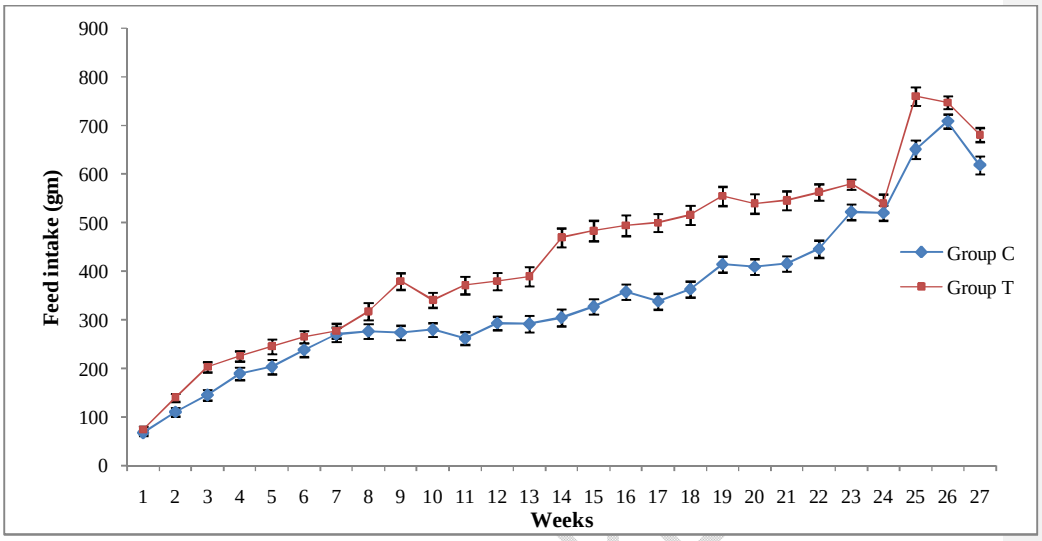

Figure 1:Average feed intake (gm) by pubertal rams in two groups (C & T)

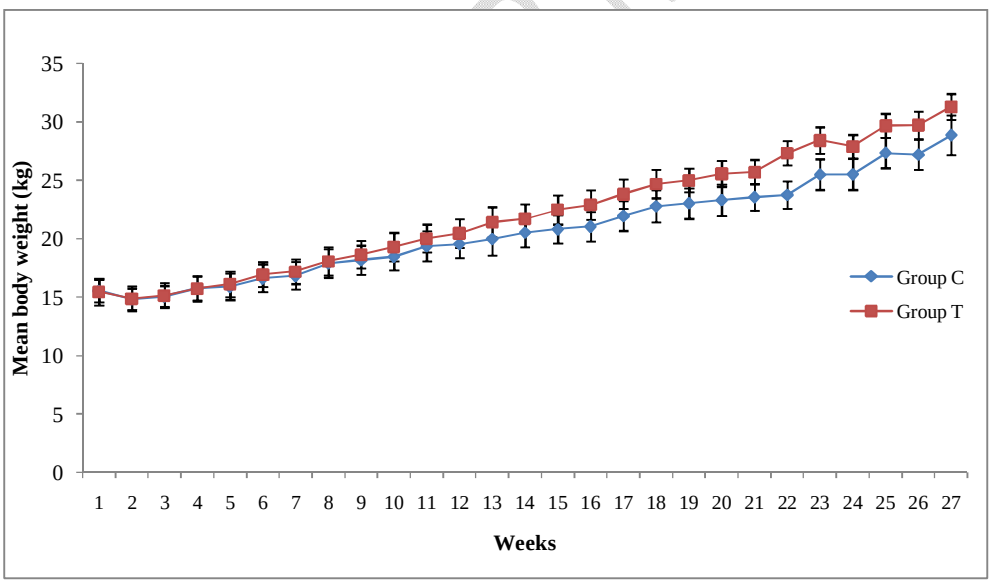

Figure 2:Mean ± SE body weight (kg) of pubertal rams in two groups (C & T)

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, circumference 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 (Rege*et al.*, 2000; Agga*et al.*, 2011). The effect of age and body condition score were significant ($P < 0.01$) on all the testicular measurements in Red Sokoto bucks (Akpa*et al.*, 2013).

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 ($p<0.05$)

Table 2: Body measurements (Mean ± SE) of pubertal ramsin 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 with different superscript with in a row vary significantly ($p<0.05$)

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.

Effect on sexual behavior: Normative sexual behaviour is one of the eight social behaviours reported in sheep (Scott, 1945), is a consequence of the interaction of various factors like physiological, neural, genetic, nutritional, climatic and age. Nutritional factors 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 behaviour 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 (Lynch *et al.*, 1992). 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 is 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 (Patel *et al.*, 2007).

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 (Simitzis*et al.*, 2006). 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 behaviour 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 (Casteilla*et al.*, 1987) and they are also expressed in the case of non-estrus ewes (Tomkins and Bryant, 1974).

Vocalisation with leg kicking and flehmen reaction was almost same in both group. 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 ejaculation 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 behaviour 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 (Martin *et al.*, 2010).

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 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 seconds in comparison to non oestrus females (Shearer and Katz, 2006), and 6.91 seconds in pubertal bucks (Kerkettaet *al.*, 2014). The variation in mean ejaculation latency depends upon the factors like breed, age, season and nutrition. Further Zarazagaet *al.* (2009) 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 (Hotzelet *al.*, 1998), resulting in the rate of production of testosterone (Setchellet *al.*1965). In contrast it's also proved that except for extreme under

nutrition, these effects are not linked to major changes in testosterone production or in sexual behaviour(Martin *et al*., 2010). 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 (Banks,1964).Surprisingly, it is also reported that it is greater in rams with smaller testes than in rams with larger testes (Raadsma and Edey,1985).Irrespective of group the time increased with advancement of mating, similar to Shearer and Katz (2006) and Kerkettae*t al*. (2014) who also reported that PEI increased with advancement of mating.

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 ($p<0.05$)

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 male in group are interested in female and also due to lack of experience showed this as extended behaviour. 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 bucks was 60.53± 1.223 seconds, in Sannen buck 64.56± 1.233 seconds (Sinha and Singh, 1982) 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 behaviour. In both, refractory period increases with

the advancement of mating upto 3rd mating, which is very obvious in mating session behaviour.

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	4thRefractory 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 ($p<0.05$)

Libido is a sexual desire to mate which refers to sexual motivation, indulge behaviors such as seeking and detecting mate, courtship, and mating (Parkinson and Vermunt, 2000). Libido is chiefly measured by reaction time, defined as the elapsed time between exposure to stimuli and first service (Chenoweth, 1999; Ott and Memon, 1980). Libido in males in measured by observing and recording various parameters like based on reaction time, number of ejaculation. Libido was average for pubertal rams in both group, 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 behaviour of lambs. Overall, it appears that libido is more sensitive to under nutrition than spermproduction (Martin *et al*., 2010). 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 group were mounting with enthusiasm of value +1. Young rams usually show low libido on introduction to a new group (Holmes, 1980). Further it was reported that rams having lack of exposure to ewes during their early life exhibited poor sexual behaviour (Price *et al*., 1994).

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 ($p<0.05$)

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±7.96vs 257.4±9.92 days).The semen volume, mass motility and sperm concentration ~~was~~ were0.61±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 ~~clearly~~ visible indicating the effect of tannin on seminal parameters. Condensed tannin as an antioxidant tool ~~split~~ splitsthe negative effect of oxidative chain reaction and improve the process of spermatogenesis, thereby enhance semen quality and general ~~testicles~~ testiclehealth (Showell*et al.*, 2014 and Asadi*et al.*, 2017).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~~tannin-fed lambs leading to improvement in body and testicular measurement.The ~~tannin rich~~tannin-rich feed may incur the maximum benefit of on sperm concentration and motility (Ahmed *et al.*, 2021) with a ~~slight~~ slightlytoxic effect on sperm cells which was ~~in accordance with~~bythe current study. Kabiraj*et al.* (2011) observed that higher testicular size ~~have~~ hashigher 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 (Sharma *et al.*, 1991).

Conclusion

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

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