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Role of flushing on some semen characteristics and copulating behavior in local black bucks

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Abstract

This study was conducted at the animal farm, department of animal science (Bakrajo field), College of Agricultural Sciences, University of Sulaimani, Kurdistan region–Iraq, to find out the reproductive performance and evaluation of semen in a local black buck and their relation to different levels of nutrition . Eighteen local black goats (6 bucks+12 does), 1.5-2 years old were used and randomly divided into two groups (3 bucks+6 does).The groups were fed two levels, 300gm/day (0.9 Mcal ME) as low-level feed and 600gm/day (1.8Mcal ME) as high-level feed for 2 months of the mating season. The live body weight (LBW), buck semen characteristics and reproduction performance was measured through this experiment. Behavior data were collected during hand–mating, which include Copulation number and the period between two copulations (C-C), the period between first meeting and copulation (M-C), the courtship behavior index value of bucks (CBIVB) and mounting behavior of buck. The results obtained revealed no differences in LBW of bucks fed with either low or high level of concentrates during flushing period. The characteristics of buck semen in local black goat were not significantly different at all period of flushing, except live sperm percentage was significantly ($P \leq 0.05$) in a high level of treatment at a period to flushing and significant improvement ($P \leq 0.05$) in color in a period after flushing. The results of buck behavior revealed no significant differences between breeding groups. But flushing decreases the reacting time and ejaculation time when matting between does fed high level (H) and bucks fed low (L) and high level (H). The period between two copulation (C-C) recorded shorter time when mating does flushing high-level feed (H) with bucks fed low (HxL: 286.66 sec.) and high level (HxH: 405.00 sec.).

Introduction

Reproductive efficiency is one of the most important economic traits in terms of livestock production. Maintaining good reproductive functions in the herd or flock is pivotal to the success of any livestock production system. So the fertility of male is an important factor in caprine reproduction since numerous does are generally bred to a single buck. Hence, male fertility evaluation prior to breeding is of paramount importance to achieve breeding success. Breeding males potential fertility can be evaluated in the field by assessment of mating ability, physical examination and a genital tract examination, and semen quality evaluation ***[1]***. These methods are useful for screening out sub-fertile males, although neither allows precise determination of the pregnancy rates that males actually achieve ***[2]***. The Sexual behavior is directed by a sophisticated interplay between steroid hormone actions in the brain that give rise to sexual arousal ability and experience with a sexual reward that gives rise to expectations of competent sexual activity, sexual desire, arousal, and performance ***[3]***. Libido is a male's desire to mate, is an important aspect of fertility, that

affected by factors such as nutrition, age, diseases and season of mating. Mounting and thrusting behavior, sniffing of the genital region and flehmen reaction (curling of the upper lip of the male in response to detecting sexual readiness of the female) are well established common behaviors of normal sheep and goats, this behavior is regulated by the release of testosterone, produced by specialized cells in the testes***[4]***. Libido is typically measured using the reaction time, defined as the elapsed time between exposure to stimuli and first service ***[5]***.

Nutrition is considered to be an important factor affecting seasonality of reproductive functions in bucks ***[6]***. In focused feeding, it was possible to improve the reproductive performance of sheep and goats by using short, targeted feeding regimes ***[7]***. So the productivity of goats is the efficient utilization of nutrients which is possible with a satisfactory supply of energy. Energy requirements are affected by age, body size, physiological state, environmental factors, hair growth, muscular activity and relationships with other nutrients ***[8]***. Flushing is generally recognized as a significant regulator of reproduction and it can be accomplished either by allowing animals to graze lush nutritious pasture or by feeding energy and protein supplements ***[9]***. The flushing in simple terms refers to exposing the animals on a higher plane of nutrition 30 days prior to breeding and 30 days after breeding to make the does gain weight and body condition, but purposeful elevation in the plane of nutrition prior to and a few weeks after mating ***[10]***. Flushing prior to the beginning of the breeding season positively affects body condition score (BCS) and improves reproductive performance of goats ***[11]***. Since nutritional requirements vary throughout the reproductive cycle, strategic feed supplementation can also be an important tool to improve reproductive efficiency. Several studies reported a decline in reproductive activities, quality and quantity of semen during the non-breeding season in male goats ***[12]***; ***[13]*** and ***[14]***. The decreases in nutrient intakes have reduced libido ***[15]***, semen volume ***[16]*** and ***[15]***, sperm output ***[17]*** and ***[18]*** of adult breeding boars. Seminal attributes are affected by many factors, including the breed, body weight, age, management, climatic conditions, nutrition, method of semen collection and degree of sexual stimulation ***[19]***. Sharma *et al.* (1991) ***[20]*** reported that the concentration sperm might vary according to variation in age, breed, collection frequency, feeding regime and climatic condition. The effect of nutritional restrictions on fertility are more notable in the female than in the male, nutritional deficiencies delay the onset of puberty and depress production and characteristics of semen in the male, nutrition affect the endocrine rather than the spermatogenesis function of the testes ***[21]***. Louis *et al.* (1994) ***[22]*** reported that when energy intake was maintained and protein intake was reduced, there was a significant reduction in libido and semen volume and a trend for reduced sperm output. The nutritional limitations affect the reproductive process in both genders, and the genders differ in the timing of maximum energy demand–in males, high energy intake boosts spermatogenesis before fertilization and provides energy reserves to support sexual and aggressive behavior during mating ***[23]***. So it was necessary to conduct this study to find out the reproductive performance and evaluation of semen in a local black buck and their relation to different levels of nutrition.

Materials and Methods

Animals and treatments: This experiment was conducted at the animal farm, department of animal production (Bakrajo's filed), College of Agricultural Sciences, University of Sulaimani, from August 1st, 2015 to March 1st, 2016. Eighteen adults local black goat (6 Bucks + 12 Does), aged 1.5-2 years and averaged 35-45 kg in weight for does and bucks, respectively were used in this experiment, they were divided into two equal groups of both sexes. Before starting, the animals were adapted 15 days for feeding regime and increasing the amount of feed gradually. Each animal was reared in individual pens (1.10×1.20cm). Moreover, all does and bucks were clinically examined by a veterinarian, vaccinated against enterotoxemia. All does be examined using Ultrasonic diagnostic instrument to make sure that all does be not pregnant. After the adaptation period of 15 days, flushing of both sexes started at low and high energy levels, for 30 days before mating (pre-mating), and continued for 30 days after mating. T_1 (low energy level) contained 0.9 Mcal ME by feeding (300 g/d). T_2 (high energy levels) contained 1.8 Mcal ME by feeding (600 g/d) (Table 1). Thereafter, all experimental does be returned to the normal diet (low energy) until kidding,

feeding animals 2% of their body weight during the gestation period and *ad libitum* hay. Animals of both sexes were weighed three times during the experiment (at the beginning, middle, and end) ***[24]***.

Table 1: Ingredient composition of the diet provided to the 60 days during flushing period.

Ingredient	***%***
Barley	*45*
Wheat grain	*13*
Corn grain	*25*
Soybean meal	*15*
Vitamin & minerals	*1*
Salt	*1*
Total	*100*
Crud protein (%)	*15.7*
**** Metabolisable energy Kcal/kg***	*3.0184*

*The nutritional requirement determined according to (NRC, 2007) ***[25]***.

Mating behavior: The does and bucks were mated at three different times on the same day (6:00 AM, 12:00 PM and 6:00 PM) for a half hour for each mating group. Animal behavior was monitored in a specially designed observation area (2 × 11)m for 30 min. Observed behavior of doe and buck that for bucks were assessed according to Thompson (1995) ***[26]***; Bianchet and Côté (2008) ***[27]***, by visual count of M= mounting, AS= Anogenital sniffing, F= Flehmen, TV= Touching of vulva, LK= Leg-Kicking, T= Tongue-put out, BT= Butting to doe, CBIVB= Courtship behavior index value of buck, = (CBIVB= M + AS + F + TV + LK + T + BT). Behavioral data were collected by hand-mating. Copulation number and the period between two copulations (C-C) were recorded and the period between the first meeting and copulation (M-C) was also registered. Libido was assessed by reaction time as described by Hoflack *et al.* (2006) ***[1]***. Reaction time was also recorded, measured as the period of time between the first contact with the teaser buck and the first false mount with the penis erected (expressed in seconds). Ejaculation time, the time between introducing the teaser doe to the buck and successful ejaculation (/sec) was measured.

Semen characteristics: Semen samples were collected three times (before, middle and after treatment) from each buck throughout the experiment using electro ejaculator (5-10 Volt, 10 sec.)(Electro jac6) Lexington, KY 40511, Made in the USA. Each sample was collected into gradual tube (15cc), and was immediately transferred to the laboratory and placed in a water bath at (37.5°C) for evaluation of the physical parameters of semen and sperm characteristics. The volume of the ejaculate was measured directly by the collection in the gradual tubes (15ml) to the nearest (0.1ml) ***[28]***. The color was assessed according to the method of Evan and Maxwell (1987) ***[29]***. Hydrogen ion concentration (pH) was measured by pH meter ***[30]***. Percent of mass activity was subjectively assessed according to Avdi *et al.* (2004) ***[28]***, by placing a small drop of fresh semen on a pre-warmed slide (37.5°C) and scored under a microscope at magnifying powers (10x) within 2 minutes of collection. Individually progressively motile sperm was subjectively assessed on an extended by dilution a drop of semen in saline solution (2.9% Sodium citrate $Na_3C_6H_5O_5$), on the warm slide (37.5°C), mounting it with a cover slide and examining it under microscope (40x objective) and scored according to Avdi *et al*. (2004) ***[28]***. Concentration was calculated with the aid of a (Haem Cytometer Chamber). The semen was diluted (1:200) with 0.9% chloride sodium and 0.01% mercuric chloride, and few drops of Eosin were added to facilitate counting the staining heads. The spermatozoa were counted under the high power objective (400x magnification). Sperm concentration was measured according

to the method of Salisbury *et al.* (1943) ***[31]***. The number of sperm /ml was calculated according to the following formula:

$$C = \frac{N}{80 \times 400 \times 10 \times 200}$$

Concentration = Number of sperm concentration /ml

N = Number of sperm in five squares.

80 = Number of the small square in the five square (5 medium square × 16 small square).

400 = 25 Medium Square × 16 small square (Total Square in slides).

200 = dilution ratio.

10 = Multiple the number of sperm in 10 to be the number of sperm in $1mm^3$ of seminal fluid.

The percentage of live sperm was carried out by placing a small droplet of fresh semen on dry pre-warm slide (37.5°C), and mixed with (2) drop of eosin stain (5%) and (1) drop of nigrosine stain (10% to stain Background) as described by Campbell *et al.* (1956) ***[32]***. Live sperm was determined by counts of (200) sperm under oil immersion (100x). The percentages of abnormal sperms were assessed according to the method of Milovanov (1960) ***[33]***. The slides prepared for counting dead and live sperm were utilized for counting abnormal sperm. The examination was done on (200) sperm at random under oil immersion (100x). Abnormal sperm includes abnormal head, tails, and cytoplasmic droplets form on the neck of spermatozoa during spermatogenesis.

Statistical analysis: Data obtained were analyzed using XLSTAT program (version-7.5, 2004) to determine the effect of different treatments and difference between them. Moreover, different models and producers were used according to the type of data obtained. Differences among treatment means were tested using Duncan's test ***[34]***. The analyses for the LBW data were analyzed as continuous independent variable with mean Mx (Covariate). The semen characteristics (volume, pH, live sperm percentage, abnormal sperm percentage, and sperm concentration) were analyzed using a Non-parametric (Mann-Whitney s test), as well as sexual behavior (ejaculation time, period between two copulation C-C, period between first meeting and copulation M-C), were analyzed using a Non-parametric (Kruskal-Wallis test).

Result and discussion

Table (2) showed the effect of flushing on live body weight (LBW) in both sexes (male and female) at middle and after treatment. Differences in LBW were not significant ($P \leq 0.05$) between low and high treatment and LBW increased from the beginning to the end of flushing in both levels of low and high level of ME which were increased from 37.71 to 40.33 kg for low and from 37 to 38.10 kg for high level for the female. In bucks, the same results were observed, which were no significant ($P \leq 0.05$) differences between the low and high treatment, and LBW increased from the beginning to the end of flushing in low level of ME which were increased from 44.51 to 47.39 kg, whereas LBW decreased from the beginning to the end of flushing in high level of ME which were decreased from 47.25 to 46.76 kg, respectively.

LBW plays an important role in determining characteristics of farm animals, especially the ones having economic importance. However, in the present study, the body weight was not different to the findings by Acero-camelo *et al.*, (2008) ***[8]***, who reported that there were no differences in body weight of does supplemented with either low or high level of concentrates during flushing period. Hafez *et al.* (2011) ***[35]*** stated that supplementation with low and high energy was not recognized on LBW during the beginning of the experiment, pre-breeding, breeding, and birth. This result may be due to the indemnity of lack energy from other resource of feeding in the low level of feeding.

Table 2: Flushing effect on live body weight (Kg) (Mean ± S.E) of both sexes in local black goat at the middle and end of the experiment.

Feeding Levels	Female		Male	
	Periods		Periods	
	Middle	End	Middle	End
Low (0.9 Mcal ME)	37.71 ± 1.20^{a}	40.33 ± 0.47^{a}	44.51 ± 2.59^{a}	47.39 ± 2.67^{a}
High (1.8 Mcal ME)	37.00 ± 1.26^{a}	38.10 ± 1.14^{a}	47.25 ± 3.73^{a}	46.76 ± 4.52^{a}

- Means with different superscripts in the same column differ significantly (P≤0.05).
- L= Low (300g/d; 0.9Mcal ME) ; H= High (600g/d; 1.8Mcal ME).

Means (± S.E) of semen characteristics obtained for the two feeding levels at the three periods throughout the experiment are presented in Table (3). Results revealed a significant difference (P≤0.05) in semen color after 8 weeks of feeding treatment, which were 5.00±0.00 and 2.90±1.10 in low and high level feeding respectively, whereas significant difference (P≤0.05) in live sperm percentage (%) in period before flushing, which were 99.83±0.16 and 96.33±0.92 in low and high level feeding. While there were no significant differences in all semen parameters between animals at the two levels of feeding. Sperm production and semen quality have been shown to vary between genotypes and to be affected by a wide range of environmental factors ***[36]***. Sharma *et al.* (1991) ***[20]*** reported that sperm concentration might vary according to variation in age, breed, collection frequency, feeding regime and climatic condition.

The bucks have the highest libido, fertility, semen quality and volume in late summer and fall which is directly correlated to the seasonal breeding pattern of does, which, during the fall, the endocrine system also increased levels of testosterone and luteinizing hormone ***[37 ; 38]***. Also, Martin *et al.* (2010) ***[39]*** in a review of the interactions between nutrition and reproduction in the male ruminant commented that a reduction in feed intake profoundly affected sperm production and that energy intake was likely to be more important than protein intake in remediation, as well the color of the ejaculate in ruminant species is generally related to sperm concentration. The yellow color is normal, it is not related to any pathological changes and semen coloration, and it is believed to be inherited ***[40]***. Sperm motion parameters are very important for oocyte penetration ***[41]***. Moreover, progressive sperm motility is essential for efficient penetration ***[42]***. Rams and bucks can experience temporary periods of poor semen quality due to stress. Higher than normal temperatures caused by weather or infection are the most common causes. Exposure to extreme environmental temperature can damage semen quality for up to 45 days ***[43]***. Accordingly, the lack of differences in sperm motility or abnormalities between treatments is consistent with other investigations in which only protein ***[17]*** or both protein and energy intakes were varied ***[16]*** and ***[15]***.

Table- 3: Flushing effect by two levels of feeding on semen characters before, middle and end of treatment (Mean ± S.E) in local black goat.

Periods	Feeding levels	Color	Volume (ml)	Mass Activity (%)	Individual Activity (%)	Concentration (X 10^7 / ml)	Live sperm (%)	Abnormal Sperm (%)	pH
Before experiment	Low (0.9Mcal ME)	3.00 ± 0.58^a	1.30 ± 0.35^a	70.00 ±20.00^a	68.33 ±26.67^a	124.00±15.31^a	99.83 ± 0.16^a	07.00 ± 1.85^a	7.05 ± 0.45^a
	High (1.8Mcal ME)	2.57 ± 0.98^a	1.00 ± 0.00^a	50.00 ±34.64^a	35.00 ±11.58^a	102.00±31.55^a	96.33 ± 0.92^b	06.06 ± 1.23^a	7.27 ± 0.11^a
Middle of experiment	Low (0.9Mcal ME)	4.67 ± 0.33^a	2.83 ± 0.44^a	90.00 ±00.00^a	88.33 ±6.67^a	127.66±34.06^a	98.16 ± 1.59^a	07.66 ± 1.64^a	7.35 ± 0.45^a
	High (1.8Mcal ME)	4.00 ± 0.58^a	2.33 ± 0.33^a	76.67 ±23.05^a	55.00 ±11.55^a	122.33±32.54^a	99.16 ± 0.16^a	09.00 ± 1.60^a	7.35 ± 0.30^a
End of experiment	Low (0.9Mcal ME)	5.00 ± 0.00^a	2.08 ± 0.22^a	70.00 ±11.54^a	48.33 ±24.03^a	118.00± 6.69^a	98.33 ± 0.92^a	12.33 ± 9.61^a	7.38 ± 0.31^a
	High (1.8Mcal ME)	2.90 ± 1.10^b	1.66 ± 0.30^a	83.33 ±6.67^a	68.33 ±26.67^a	129.66 ±5.33^a	98.83 ± 0.44^a	13.5 ± 6.72^a	7.61 ± 0.16^a

- Means with different superscripts in the same column with the same period differ significantly (P≤0.05).
- L= Low (300g/d; 0.9Mcal ME) ; H= High (600g/d; 1.8Mcal ME).

In this study, results of mating behavior traits of local black bucks are presented in Table (4). However, it also shows the mating behavior (M), Anogenital sniffing (AS), Flehmen (F), Touching of the vulva (TV), Leg-Kicking (LK), Tongue-put out (T), Butting to doe (BT) and Courtship behavior index value of a buck (CBIVB). The results of buck's behavior show no significant differences between breeding groups in mating behaviors. Such results are in agreement with those reported by ***[44]*** and ***[45]***. This may due to the equal sexual ability of bucks that live together, which is why the behavior to acquire among themselves, so there are no significant differences between mating groups in mating behaviors ***[46]***. Or maybe due to the reason generate jealousy between bucks and starts buck can stimulate the companion with him ***[47]***.

Table 4: Flushing effect on sexual behavior parameters (Mean ± S.E) of local black bucks.

Mating groups (F × M)	Bucks Sexual Behavior Parameters							
	M	AS	F	TV	LK	T	BT	CBVIB
L × L	10.33 ± 06.39^a	3.33 ± 2.03^a	2.00 ± 1.15^a	07.66 ± 4.09^a	05.33 ± 3.18^a	6.66 ± 3.38^a	0.66 ± 0.67^a	34.66 ± 18.89^a
L × H	23.00 ± 10.26^a	7.33 ± 3.28^a	1.66 ± 1.20^a	17.66 ± 7.33^a	11.00 ± 4.50^a	9.00 ± 3.51^a	0.33 ± 0.33^a	69.33 ± 29.28^a
H × L	14.33 ± 05.61^a	4.66 ± 1.76^a	2.33 ± 0.88^a	07.66 ± 3.67^a	07.33 ± 2.96^a	4.66 ± 1.76^a	1.33 ± 0.33^a	39.66 ± 15.21^a
H × H	30.00 ± 15.13^a	9.66 ± 4.81^a	4.66 ± 1.20^a	17.00 ± 5.51^a	15.33 ± 7.69^a	9.66 ± 3.84^a	2.33 ± 1.20^a	84.00 ± 37.99^a

- The parameters for all groups mating were not significant differences (P≤0.05).
-M, mounting; AS, Anogenital sniffing; F, Flehmen; TV, Touching of the vulva; LK, Leg-Kicking; T, Tongue-put out; BT, Butting to doe and CBIVB, the Courtship behavior index value of the buck.
-F= Female and M= Male.
-L= Low (300g/d; 0.9Mcal ME) and H= High (600g/d; 1.8Mcal ME).

The descriptions of mating behavior times are shown in Table (5). In this study, behaviors of doe and buck were used as an indicator of their courtship with each other. The time was recorded to describe means of reaction time, ejaculation time and period between two copulation that mated between feeding levels groups. The flush feeding reduces the Reaction time when mating between bucks at low feeding and mating those bucks with does at low feeding level (344.33 and 453.33 Sec., respectively). The ejaculation time when matting between bucks of high feed level with does at high and low feeding levels, was 280.00 sec. and 150 sec. respectively. Moreover, the period between two copulation (C-C) recorded low time when mating does at flushing high-level feed with bucks fed low and high level, were 286.66 and 405.00 sec. The best reaction time in H×L may be due to the effect of high level in female and low level in a male of nutrition that causes more active of bio-operations in the body including sexual activity and increasing sex hormone thus increased the sexual case ***[48]***. The effect on ejaculation time of animals may be due to the role of pheromones. They are chemicals capable of acting outside the body of the secreting individual to impact the behavior of the receiving individual which released from female (doe) that stimulate the sexual case in male goats so showing the increase of the ejaculation time in the group ***[49]***. The decrease in the period of two copulations that recorded in does high-level feeding may be due to the short period of mating when the bucks inserted to mating in the group and may also be the does were at a high level of BCS that affecting on mating comparing to low BCS of other groups.

Table 5: The descriptive statistics for the behavior times (Sec.) mating groups between both sexes at different feeding levels.

Parameters	*Mating groups (F × M)*	*$\bar{X}$*	*SD*	*Min.*	*Max.*
Reaction time (Sec.)	*L×L*	*453.33*	*411*	*60*	*880*
	L×H	*670.00*	*96*	*600*	*780*
	H×L	*344.33*	*44*	*300*	*388*
	H×H	*553.33*	*562*	*180*	*1200*
Ejaculation time (Sec.)	*L×L*	*640.00*	*302*	*360*	*960*
	L×H	*280.00*	*92*	*180*	*360*
	H×L	*570.00*	*689*	*10*	*1340*
	H×H	*150.00*	*90*	*60*	*240*
C-C (Sec.)	*L×L*	*680.00*	*125*	*540*	*780*
	L×H	*600.00*	*375*	*180*	*900*
	H×L	*286.66*	*153*	*120*	*420*
	H×H	*405.00*	*15*	*390*	*420*

- L, Low feeding level (0.9 Mcal ME); H, High feeding level (1.8 Mcal ME):
- C-C, Period between two copulation ; $\bar{X}$, the mean of matting group ; SD, Stander Deviation ; Min., minimum ; Max., Maximum.

Conclusion

There were no significant differences in LBW in both sexes feeding with either low or high level of concentrates during the flushing period. There were no significant differences in all semen parameters after using the two levels of feeding in local black bucks, while a significant difference ($P \leq 0.05$) in semen color after 8 weeks of feeding treatment and live sperm percentage was found. Whoever, no significant differences between the mating groups in mating behaviors of bucks were found. But the flush feeding reduces the reaction time and ejaculation time when matting between does feeding high level and bucks feeding low

level. Otherwise, the period between two copulation (C-C) recorded shorter time when mating does on the high level with bucks at low and high levels.

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