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Effect of silage feeding on post thaw semen quality in Jamunapari buck

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Abstract

A study was conducted to evaluate the effect of silage feeding as replacement of green on seminal attributes in fresh and frozen thaw semen. During the experiment 18 bucks of similar age, weight and semen quality maintained in individual pen at semen production station, DUVASU were selected for experiment. The animal were divided into 3 groups each comprising 6 bucks. The bucks in control group were fed with complete green, group II with green and silage and in group III the green was completely replaced by silage. The animal under the trial were maintained in individual pan through experiment period of 90 days. The semen was collected from each buck twice a week using artificial vagina. A total of six ejaculate were collected from each buck. The semen collected from buck of each group was pooled to minimize individual variation. The pooled semen was evaluated and later diluted with Tris- glycerol-egg yolk extender, equilibrate and subjected to freezing. The frozen semen was later evaluated for post thaw semen quality. The evaluation of result exhibited no significant difference in control group feed with green when compared to the test group with green and silage and silage alone. The parameters recorded for the motility, path velocity and kinematic character (curvilinear velocity (VCL, $\mu\text{m}/\text{sec}$) (average path velocity (VAP, $\mu\text{m}/\text{sec}$), straight line velocity (VSL, $\mu\text{m}/\text{sec}$), Linearity (Lin%), Straightness (Str %), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement (ALH, μm) were well within the normal range with no significant difference. So, it may be concluded that replacement of green with silage do not have any negative effect on semen quality of breeding bucks maintained under intensive feeding.

Keywords: Goat, post thaw quality, semen, seminal attributes, silage

Introduction

Artificial insemination (AI) in goat is a new upcoming assisted reproductive technology for genetic upgradation. This technology is being widely adopted by different states across India. But with wide variability in goat breed, fewer goat frozen semen stations, increasing demand of frozen semen doses and limited capacity to maintain the breeding buck at semen station, it becomes essential to optimize the available resources for its judicious use.

The production capacity of semen station largely depends upon the quality and volume of semen ejaculate collected from individual buck. Traditionally, bucks are being reared on extensive feeding system with free access to green fodder and are allowed for indiscriminate breeding. But with establishment of semen station that requires a standard protocol for buck rearing, breeding bucks at are to be maintained individually under intensive feeding system. Since this system of intensive feeding is new, required detailed study on various managemental aspects of animal rearing.

Feeding and housing management of buck are two main prerequisite that determines the health, productivity and reproduction efficiency of animals (Dalmau *et al.*, 2020) [1]. Reproduction potential of buck maintained at semen station is generally assessed by the volume and quality of ejaculate (Tanga *et al.*, 2021) [2]. Feeding management includes the balanced diet along with all the essential nutrient required for health and production. The diet of ruminant comprises of concentrate, green, dry fodders and mineral supplementation. Nutrients need for growth and development include minerals, vitamins, and hormones are essential components of many enzymes, cofactors in regulate immunity, and reproduction health.

Even moderate deficiencies of minerals can adversely impact animal performance (Singh *et al.*, 2019; Kesari *et al.*, 2022) [3] [4]. Green fodder is the component of animal diet that varies with season. Frequent variation in the green fodder not only affect the rumen microbiota but also induce metabolic stress reflected through indigestion especially in small ruminants (Guo *et al.*, 2021) [5]. Nutritional stress affects the health, productivity and even reproductive performance of animal in terms of libido, semen volume, concentrate and quality (Harrison *et al.*, 2022) [6]. Even a greater intake in energy levels can decrease ejaculate volume, sperm concentration, motility, and morphology of the sperm (Perkovic S, 2001) [7] as well as create a hormonal imbalance (Singh *et al.*, 2018) [8] all of which can impact male fertility.

Maize silage is considered to be the viable alternate with better digestibility and nutritive value (Anesio *et al.*, 2017) [9]. Replacement of green with silage can be alternate to minimize periodic change in green fodder made available to bucks with more acceptability availability throughout the year. So, the present study was designed with an objective to evaluate the effect of silage feeding on semen production and post thaw semen quality of breeding buck.

Materials and Methods

Experimental design

The study was conducted to evaluate the effect of maize silage feeding on the reproduction performance of breeding bucks maintained at goat frozen semen station, DUVASU, Mathura. Eighteen healthy breeding bucks of Jamunapari breed (2.5-3 years of age) were selected from the herd maintained at breeder farm. Experimental bucks were randomly assigned into three groups; Control (C), Treatment 1 (T)-1 and Treatment 2 (T)-2 with six animal each on basis of body weight. The bucks of each experimental group were maintained and fed individually on roughage and concentrate based ration to meet out requirement as per ICAR (2013) feeding standard. Diets were prepared by taking concentrate and roughage in the ratio of 40:60 respectively. The roughage part composed of gram straw (40%) and corn silage/green fodder (20%). All the animals in control group were fed with basal diet comprised of concentrate mixture, gram straw and green fodder. The animals in the treatment groups (T)-1 were fed with similar feeding regime except the green fodder, in which 50% of green fodder was replaced with corn silage and in (T)-2 group 100% of green fodder was replaced with corn silage. The duration of experimental period was of 90 days. Semen was collected twice a week from each buck using artificial vagina. A total of six ejaculates from each buck were collected in 21 days after completion of feeding trial. The ejaculates of individual buck within group were pooled to reduce individual variation. Pooled semen of each group was evaluated for the seminal attributes *viz.* mass motility, concentration, volume and pH and later subject to process of semen cryopreservation. To perform semen cryopreservation, the pooled sample was diluted to final concentration of 400 million spermatozoa per ml using a tris-based egg yolk-glycerol semen extender and incubated at 4 °C for period of 4 hrs. In cold handling cabinet. The equilibrated semen was cryopreserved using semen programmable freezer and finally transferred to LN₂ container maintained at -196 °C. To evaluate the effect of silage feeding on post thaw semen quality different attributes indicative of sperm health were evaluated using CASA and flowcytometry. Viability, acrosomal integrity, membrane fluidity of sperm and ROS affected spermatozoa were evaluated using flow cytometer.

To evaluate the frozen thaw semen dose (250 µl), semen from straw was transferred in 1.5 ml plastic tube and mixed with 500 µl of EBB solution as stock sample solution. The stock solution was utilized to evaluate different seminal attributes using easy kits specific for each seminal attribute. The motility parameters were evaluated through computer assisted semen analyzed. The semen sample was diluted with 750 µl of semen extender to reach the final concentration of 100 µl/ml and mixed gently. 10 µl of the mixed sample was placed on 8 chambered Leza slide and examined under 10x objective through CASA using specific software.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The means were compared using Analysis of Variance and presented as mean ± standard error (SE).

Result and Discussion

During the experiment, the effect of silage feeding was studied on reproductive performance of breeding buck maintained under intensive system. The breeding buck was evaluated in neat and cryopreserved semen. The values recorded for volume, concentration, mass motility and progressive motility in the fresh ejaculated semen was non-significant among all the three groups during the experiment (Table 1). A non-significant difference in the seminal attributes of test group may be attributed to availability of sufficient nutrients supplied by silage to meet the nutrient requirement as was diet offered with green in control. The examination of frozen thaw semen samples for various seminal attribute in test and control group has been presented in Table 2. During the experiment, a non-significantly difference in various seminal attributes were recorded in (T)-1 group feed with green and silage and (T)-2 group with animal solely maintained on silage as replacement to green when compared to the control group with green fodder as constituent of diet. Non-significant value in test and control group may be attributed to better digestibility of corn silage with all essential nutrients available as an alternate to green.

The values for the acrosomal integrity and reactive oxygen species (ROS) negative spermatozoa were non-significantly test groups compared to control. The reason for non-significant difference in the test group may be attributed to maize as an alternate energy source with all the essential nutrients at par to green fodder required for rumen function and energy metabolism (McGrath *et al.*, 2018) [10]. Although feeding silage with antioxidants supplementation has been reported to give better results in terms of improved antioxidative status. Tian *et al.* (2019) [11] recorded an increase in the antioxidative status of lactating dairy goats feed on anthocyanin-rich purple corn stalk silage which was not supplemented in present study.

Membrane fluidity is the characteristic of sperm membrane that maintains the internal environment of spermatozoa (Maitan *et al.*, 2021) [12]. During the experiment, non-significantly values for sperm plasma membrane fluidity were observed in test and control group. Level of antioxidant in seminal fluid is a function of nutrient available in diet of animal. Antioxidant play an important role in regulating the ROS level in seminal plasma (Walczak-Jedrzejowska *et al.*, 2013) [13]. An imbalance in the ROS production and neutralization increases the ROS load in seminal plasma. Increase ROS level leads to efflux of membrane cholesterol

and phospholipids leading to membrane destabilization (Wang *et al.*, 2017) [14]. During the experiment, test groups which was supplement with green and corn silage, corn silage as replacement of green fodder might have made available all the essentially required nutrients as are available in green fodder thus maintaining the required antioxidant level in seminal plasma, thus protecting spermatozoa from oxidative damages and membrane damage that resulted in non-significant difference among the test groups and control. To further confirm the findings of the experiment, the seminal plasma was evaluated for SOD and Catalase. It was observed that the test groups supplemented with green and silage and silage alone had non-significant differences in values of SOD and catalase indicating that corn silage can maintain the same level of antioxidants in seminal plasma. The motility is considered as most important seminal attribute contributing to successful conception. During the experiment the motility and

motility pattern curvilinear velocity (VCL, $\mu\text{m}/\text{sec}$) (average path velocity (VAP, $\mu\text{m}/\text{sec}$), straight line velocity (VSL, $\mu\text{m}/\text{sec}$), Linearity (Lin%), Straightness (Str %), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement (ALH, μm) indicative of sperm health was non-significantly among the test and control. Sperm motility is the parameter that exhibit change in pattern under stress. Seminal plasma with low concentration of antioxidant exhibits higher proportion of sperm with ROS plasma membrane and acrosomal damages (Takeshima *et al.*, 2020, Anand *et al.*, 2017, Anand and Yadav, 2016) [15, 16, 17]. Any change in the structural integrity of sperm cell may lead to change in motility patterns. Non-significantly values motion character and kinematic character in semen is indicate the capacity of corn silage to maintain the reproductive function thus maintain the motion character in semen as are exhibited by animal feed solely on green fodder.

Table 1: Effect of feed with green, silage and their combination on seminal attributes of spermatozoa in fresh semen

Group	Volume (ml)	pH	Mass Motility (0-5 scale)	Concentration (million/ml)	% Viability
Green fodder	0.73±0.04	6.76±0.01	4.06±0.11	4158.75±46.00	92.25±0.49
Green+ Silage	0.66±0.04	6.78±0.02	4.25±0.09	4013.12±44.19	91.75±0.81
Silage	0.67±0.03	6.75±0.02	4.31±0.09	3954.87±41.58	91.75±0.81

Non -significant difference in mean values recorded at ($p \leq 0.05$)

Table 2: Effect of feed with green, silage and their combination on seminal attributes, motility, path velocity and kinematic character of spermatozoa in post thaw semen.

Group	Green fodder	Green+ Silage	Silage
% Viability	54.29±1.84	52.86±1.61	55.57±1.47
% Polarize Mitochondria	45.29±0.80	45.71±1.12	45.50±0.75
% intact acrosome	39.00±1.09	36.15±1.22	40.43±0.86
% ROS + ve sperm	14.86±1.28	15.87±1.12	14.57±1.27
Normal membrane fluidity	43.01±1.02	41.23±0.98	46.01±1.11
Catalase (CAT) (mM H_2O_2 utilized/min/mg.protein)	12.07± 0.19	12.455± 0.18	12.11± 0.18
Superoxide dismutase (SOD)(U/mg protein)	15.08± 0.63	14.78±0.49	15.21± 0.41
% Total motile	49.71±1.37	48.43±1.11	50.71±1.26
% Progressive	33.71±0.65	34.61±0.68	35.57±1.08
VCL ($\mu\text{m} / \text{sec}$)	91.57±3.38	84.00±3.33	92.57±2.81
VAP ($\mu\text{m} / \text{sec}$)	56.43±1.39	50.71±2.52	56.14±1.29
VSL ($\mu\text{m} / \text{sec}$)	48.57±0.97	44.57±0.94	48.17±1.25
LINEARITY (%)	25.86±0.96	25.50±0.97	26.29±0.86
STR (%)	41.29±1.01	41.20±1.28	42.43±1.12
Max ALH (μm)	3.71±0.18	3.43±0.20	3.71±0.18
BCF (hz)	18.86±0.63	18.29±0.83	19.00±0.69

Non -significant difference in mean values recorded at ($p \leq 0.05$)

Conclusion

So, it can be concluded that feeding of corn silage either alone or in combination with green fodder do not have any negative impact of reproductive system of male buck, hence corn silage feeding can be an alternate for green fodder, with year round availability in diet of breeding bucks maintained at frozen semen station for semen production.

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