

IMPACT OF POLYHERBAL MIXTURE ON REPRODUCTIVE TRAITS OF ASSAM HILL GOAT

ABSTRACT

In this study, 36 Assam Hill goats were chosen and randomly split into three groups. A mixture of herbal feed was supplemented to the treatment groups @ 1 and 2 grams per kilogram of body weight, starting from 4 months old until they were 12 months old. The mean average age (days) at first heat were 199.833 ± 3.842 , 183.333 ± 2.985 and 183.167 ± 4.110 for goats in C₀, T₁ and T₂, respectively. The highest mean semen volume (ml) was found in T₂ group at 12th month 0.627 ± 0.008 , Similarly, live sperm (%) 85.355 ± 0.895 was found in T₂ group at 12th month. The overall semen qualities were found better in T₂, followed by T₁ and C₀. There was improvement in reproductive traits such as age at first heat, heat duration and age at sexual maturity in females as well as semen quality in males

Keywords: Polyherbal, supplement, Reproductive traits, Assam Hill goat

1.Introduction

Goat is the earliest domestic species, with evidence indicating domestication took place around 7000 years B.C. in Southeast Asia, near the current borders of Iraq and Iran (Mason, 1981). They are raised for multiple purposes of which milk and meat being the primary ones. The consumption of goat milk by human dates back to the initial stages of domesticating animals of economic significance (Dubeuf, 2005). The global importance of goats as vital sources of meat and dairy nutrition has been explored and documented in many recent proceedings of national and international conferences (Haenlein, 2001; Haenlein and Fahmy, 1999; Morand-Fehr and Boyazoglu, 1999; Rubino et al., 1999; Gruner and Chabert, 2000).

Medicinal plants are renewable natural resources. Traditionally some herbs or their mixtures are used to enhance animal performance through their flavouring and medicinal properties. Besides, they are less expensive and **safe** than the chemical additives as side effects are rarely observed. Careful selection of combination of herbal plant extracts may allow the manipulation of rumen microbial fermentation (Busquet et al., 2006). Certain herbs like fenugreek (*Tirigonella foenum-graecum*), and jivanti (*Leptadenia reticulata*), contain galactagogue which improve milk production if given in a suitable mixture for few days to lactating animals (Mudgal et al, 2003). Out of many herbal product Shatavari root, Fenugreek seed, Ajwain seed are known to have better production performances (Cullen et al., 2005). In animals, Shatavari have mammogenesis, lactogenesis, galactopoiesis role. Its usage reduces stress as it has antistress properties, maintains regular reproductive cyclicity due to estrogenic properties of Shatavari extracts. According to the last two livestock census reports (19th and 20th livestock census, Department of Animal Husbandry & Dairying), the number of goats in Assam has dropped sharply by 30.05%, falling from 6.169 million to 4.315 million. It is now high time to undertake research and development program to check this decline and increase the population through improvement in their reproductive ability in a sustainable manner. Therefore, the current study was aimed to study the effect of polyherbal feed supplementation consist of Shatavari root (*Asparagus racemosus*), Fenugreek seed (*Trigonella foenum-graecum*) and Ajwain seed (*Trachyspermum ammi*) on reproductive parameters of Assam Hill Goat.

2.MATERIALS AND METHODS

A total of 36 healthy Assam Hill kids (18 males and 18 females) with similar body weight were selected from the farm flock of Goat Research Station, Assam Agricultural University, Burnihat. They were randomly divided into three groups of 12 (6 males and 6 females each) animals: Group I (Control, C₀), Group II (Treatment, T₁), and Group III (Treatment, T₂). All groups were fed with same basic diet under semi-intensive care, but T₁ and T₂ received a daily polyherbal supplement at 1 g/kg and 2 g/kg body weight, respectively. The supplement contained equal parts of Shatavari root (*Asparagus racemosus*), Fenugreek seed (*Trigonella foenum-graecum*) and Ajwain seed (*Trachyspermum ammi*) powder (Figure 1). The regular diet consisted of a mix of roughage and concentrates at a ratio of 2:1 on dry matter basis to fulfil the nutrient requirement of kids, following ICAR (2013) guidelines. The roughage contained Para (*Bracharia mutica*) and Napier (*Pennisetum purpureum*) grasses mixed equally (50:50 DM basis).



Figure 1: SHATAVARI ROOT POWDER, FENUGREEK SEED POWDER AND AJWAIN SEED POWDER

2.1 Reproductive Parameters

2.1.1 In female

- a) **Age at first heat (puberty):** The day on which females showed the signs of heat for the first time was recorded.
- b) **Duration of the first heat:** The duration of time for which the female showed signs and symptoms of heat was recorded and expressed in hours.
- c) **Age at sexual maturity:** The age at which animals attained 60-70% of their adult body weight and sexually fit were recorded.

2.1.2 In male

A routine semen examination was done for semen quality evaluation for the following traits. Semen was collected using an artificial vagina (Figure 2) (Evans and Maxwell, 1987).

- a) **Volume:** The volume of fresh semen was recorded from the graduated mark of the semen collecting tube.
- b) **Colour:** The colour of the semen was recorded in the collecting tube immediately after collection by the naked eye. The colour was scored on 5 point scale (Jainudeen, *et al.* 1993).

Consistency	Grading
Creamy-grainy	5
Creamy	4
Thin creamy	3
Milky white	2
watery-cloudy	1

- iii) **Mass activity:** A drop (5-8 μ l) of semen was placed on a pre-warmed (38° C) glass slide without a cover slip and examined under a light microscope at low magnification (100X). The mass activity was scored on 5 scales (Evans and Maxwell, 1987).

Mass activity	Grading
Dense, very rapidly moving waves and eddies	5
Vigorous movement with moderately rapid waves and eddies	4
Small, slow-moving waves	3
Weak motion without forming any waves	2
No perceptible motion	1

- iv) **Individual motility:** A drop (10 μ l) of semen was placed on a clean pre-warmed (38 °C) glass slide and covered with a coverslip. The motility was determined by eye estimation seeing the proportion of spermatozoa moving progressively straight forward at higher magnification (200X) (Evans and Maxwell, 1987).
- v) **Live Sperm:** The percent of live sperms was determined using the Eosin Nigrosin staining technique with the standard method (Blom, 1950)
- vi) **Dead Sperm:** The percent of dead sperms was determined using the Eosin Nigrosin staining technique with the standard method. (Blom, 1950).



Figure 2: EJACULATED SEMEN WITH ARTIFICIAL VAGINA



Figure.3: SLIDE SHOWING LIVE AND DEAD SPERM

3.RESULTS AND DISCUSSION

3.1 REPRODUCTIVE TRAITS

3.1.1 Age at first heat, fifth heat and duration of heat

Reproductive traits included were age at first heat, fifth heat and durations of heats. The results observed during the experiment were shown in Table 1. The mean average age (days) at first heat were 199.833 ± 3.842 , 183.333 ± 2.985 and 183.167 ± 4.110 for goats in C_0 , T_1 and T_2 , respectively. And at fifth heat were 283.845 ± 3.842 , 267.287 ± 2.982 and 267.062 ± 4.179 , respectively. The goats in T_2 and T_1 were came to heat earlier than that of C_0 group. The duration (hr) of first heat were 26.087 ± 0.582 , 28.562 ± 0.970 and 28.627 ± 1.219 for goats in C_0 , T_1 and T_2 , respectively. Similarly, for fifth heat duration (hr) were 26.955 ± 0.436 , 32.317 ± 1.318 and 34.197 ± 1.010 for goats in C_0 , T_1 and T_2 , respectively. Goats in T_2 had higher duration of heat time followed by the goats in T_1 and C_0 and their analysis of variance were presented in Table 2. There were variations in the duration of heat time, its significantly high ($P>0.01$) in fifth heat but not significant in first heat duration. It was observed that the age at

first heat were significantly earlier ($P<0.01$) in T₂ followed by T₁ compared to control (C₀) group.

The results of the present study depicted that application of polyherbal supplementation in both the treatment T₁ and T₂ group had higher as well as better reproductive performance compared to control (C₀) group. The results were in agreement with Kassem *et al.* (2006) findings. Similarly, with Berhane (2000), who reported that highest reproductive performances (onset of estrus, pregnancy rate) were recorded in dairy cows supplemented with gur, linseed oil and fenugreek mixtures as compared to the control group which was without supplementation. Rajkumar *et al.* (2008) studied the effect of *Saraca asoca* stem bark and *Trigonella foenum-graecum* seeds on reproductive performance, serum progesterone and micro minerals profile in anoestrus cows and reported that the percentage of animals induced in oestrus and overall pregnancy rate using fenugreek seeds were higher (83.33 and 80.00 %) as compared to *Saraca asoca* (66.66 and 50.00 %) at doses of 50g and 100 g level.

The improvements in reproductive performance achieved by supplementing polyherbal combination @ 0.125 and 0.250 g/kg BW could be due to stimulation of the reproductive process by the steroidal and neurotransmitters effects of saponins contained in supplementation (Hussein and Badr, 2008). Estrogenic property of shatavari, one of the polyherbal ingredient, which stimulate the ovarian function, improves uterine tonicity thus helps in early uterine involution which consequently results into early initiation of estrus cycle (Pandey *et al.*, 2005). Mitra *et al.*, (1999) also reported that shatavari based herbal formulation did not possess oxytocin like activity which might be useful in uterine hypermotility associated early abortion.

The onset of puberty in goats typically occurs at 6–8 months of age in does and 4–6 months in bucks. The estrous cycle in goats is 21 days, on average but the age at onset can vary between breeds and is influenced by environmental factors, such as the season of birth, breed, geographic location (ie, latitude and climate), herd social structure, photoperiod and nutritional status. In temperate regions, spring-born animals will undergo puberty at a younger age simply because physiologic maturity coincides with periods of decreased day length. Though some breeds, such as Pygmy goats, may reach puberty as early as 3 months of age, breeding should be delayed until the animal has reached at least 60% of its mature body weight to achieve higher conception rates and safer parturition, that is why in the present study fifth estrus was considered.

The mean duration of standing estrus has been reported to be 36 hours, although it can range from 24–48 hours depending on age, breed, season, and presence of a male. Breed-specific mean estrus duration has been reported for Mossi (20 hours), Angora (22 hours), Creole (27 hours), French Alpine (31 hours), Boer (37 hours), Nubian (42 hours) and Matou (58 hours) goats. In the present study, the average mean duration (Assam Hill goat) at fifth estrus was 31.156 ± 0.921 . Estrus detection is based on behavioral signs, bleating, flagging of the tail, reddened vulva, vaginal discharge (which causes the tail hairs to stick together), and occasional “riding” by other does.

The present was similar with Jamra (2012) who observed the average age at puberty of Sahiwal heifers in control and treatment group was 739.66 ± 19.17 and 713.60 ± 16.10 , respectively. The average age at puberty of Sahiwal heifers of treatment group was significant at 5% level of significance.

TABLE 1 : AVERAGE (MEAN \pm SE) AGE AT HEATS AND DURATION OF HEAT OF ASSAM HILL GOAT OF DIFFERENT TREATMENT GROUPS

Female puberty	C ₀	T ₁	T ₂	Overall
Age at first heat (Days)	199.833 ± 3.842^a	183.333 ± 2.985^b	183.167 ± 4.110^b	188.778 ± 3.646
Duration of first heat (Hrs)	26.087 ± 0.582	28.562 ± 0.970	28.627 ± 1.219	27.759 ± 0.924
Age at fifth heat (Days)	283.845 ± 3.842^a	267.287 ± 2.982^b	267.062 ± 4.179^b	272.731 ± 3.614
Duration of fifth heat (Hrs)	26.955 ± 0.436^a	32.317 ± 1.318^b	34.197 ± 1.010^c	31.156 ± 0.921

Means with similar superscript in a row or column do not differ significantly among themselves

TABLE 2 : ANOVA FOR AVERAGE AGE AT HEATS AND DURATION OF HEAT OF ASSAM HILLGOAT OF DIFFERENT TREATMENT GROUPS

Parameters	Source of Variation	df	SS	MS	F
Age at first heat	Treatment	2	1100.111	550.056	6.779**
	Error	15	1217	81.133	
Age at fifth heat	Treatment	2	1179.111	589.556	7.808**
	Error	15	1132.667	75.511	
Duration of first heat	Treatment	2	25.163	12.581	2.275
	Error	15	82.961	5.531	
Duration of fifth heat	Treatment	2	169.447	84.724	14.372**
	Error	15	88.427	5.895	

**Highly significant ($p < 0.01$)

3.1. 2 Semen quality

In the current study, routine examination of the semen samples were done to assess the Mean volume (ml), Colour (1-5 scale), Mass activity (1-5 scale), Individual motility(%), Normal **Sperm (%)**, Live Sperm (%) and Dead Sperm (%) at 11th month and 12th month and were presented in Table 3 along with their analysis of variance were presented in Table 4, which showed significant differences ($P < 0.01$) among the treatment groups. The highest volume (ml) was found in T₂ group at 12th month 0.627 ± 0.008 , Similarly, live sperm (%) 85.355 ± 0.895 was found in T₂ group at 12th month. The overall semen qualities were found better in T₂, followed by T₁ and C₀. In the treatment group, the buck could be used for mating or even for collection of semen from 11th month onwards instead of 12th months as level of testosterone hormone (ng/ml) was well within the optimum range and body condition score was found suitable. It was observed that the improvement of semen quality in the treatment groups T₁ and T₂ were due to the shatavari root powder, fenugreek seed powder and ajwain seed powder mixture supplementation and their synergistic effect.

The bucks are generally used for mating from 12th months of age, but the present study revealed it could be used from 11th months onward provided proper plane of

management, feeding and nutritional status supplemented with herbs having property to improve testosterone hormone level resulted production of good semen qualities. The other factors that can vary semen qualities were environmental factors, such as the season of birth, breed, geographic location, herd social structure and photoperiod.

Gangwar *et al.* (2018) observed in their study that after first day of preservation, the experimental groups were having similar total sperm motility, whereas on the subsequent days, total sperm motility was significantly ($P < 0.05$) higher in Shatavari extract supplemented groups in comparison with the control group. However, significantly ($P < 0.05$) enhanced sperm motility was observed in group III containing 125 mg of Shatavari aqueous extract per 100 ml of diluter. Thus, it was confirmed the buck spermatozoa were more motile for longer duration in the group with shatavari extract. Hence, it was concluded that Shatavari helped in maintaining the sperm motility for longer duration. Similarly, after first day of preservation, all the experimental groups showed similar sperm viability, whereas on the subsequent days, sperm viability percentage was significantly ($P < 0.05$) higher in Shatavari extract supplemented groups in comparison with the control group. Hence, Shatavari was seen to help in maintaining the sperm viability for longer duration. Which was in agreement with the results of present study.

Semen volume also varies with season. The semen volume is high in spring and autumn (the reproductive season) and lower in summer and winter. The present research finding was consistent with that of Elsharif and Makawi (2004) and Gubartallah (2004). Also the results are comparable to those of bucks from different goats' breeds (Wang *et al.*, 2014). Earlier, studies reported that addition of antioxidants in semen diluter improves the semen quality (Gangwar *et al.*, 2014, Gangwar *et al.*, 2015, Gangwar *et al.*, 2018). But now a days people are trying natural antioxidants in semen extenders (Wen *et al.*, 2019) and extract from *Asparagus racemosus* (Shatavari) roots have been used in many investigations as antioxidants (Goyal *et al.*, 2003). Similar to our findings, Wen *et al.* (2019) reported that sperm motility, acrosomal integrity, mitochondrial membrane potential, plasma membrane integrity, total antioxidative activity in the 30 mg/L GSPE (Grape seed procyanidin extract) group was significantly enhanced, whereas MDA content was lower than as compared to the control group ($P < 0.05$). They also reported that AI results with GPSE supplemented semen showed higher litter size as compared to the control group ($P < 0.05$). Similarly, Zhao *et al.* (2009) reported the significant correlation between *Rhodiola sacra* aqueous extract and concentrations of GSH and MDA in frozen-thawed boar semen.

TABLE 3: AVERAGE (MEAN±SE) VOLUME, COLOUR, MASS ACTIVITY, INDIVIDUAL MOTILITY, NORMAL **SEPRM, LIVE SPERM, DEAD SPERM COUNT OF ASSAM HILL GOAT OF DIFFERENT TREATMENT GROUPS**

Semen evaluation	Age at evaluation	C ₀	T ₁	T ₂	overall
Volume (ml)	11th month	0.383 ±0.011 ^a	0.535 ±0.008 ^b	0.560 ±0.006 ^b	0.493± 0.008
	12th month	0.448 ±0.007 ^a	0.610 ±0.010 ^b	0.627 ±0.008 ^b	0.562± 0.008
Colour (1-5 scale)	11th month	3.333 ±0.067 ^a	4.133 ±0.061 ^b	4.217 ±0.031 ^b	3.894± 0.053
	12th month	4.033 ±0.080 ^a	4.483 ±0.040 ^b	4.483 ±0.031 ^b	4.333± 0.050
Mass activity (1-5 scale)	11th month	3.633± 0.076 ^a	4.033 ±0.021 ^b	4.217 ±0.031 ^c	3.961± 0.043
	12th month	4.117 ±0.040 ^a	4.300 ±0.026 ^b	4.417 ±0.048 ^c	4.278± 0.038
Individual motility (%)	11th month	75.948±0.349 ^a	80.158±0.187 ^b	81.792±0.391 ^c	79.299±0.309
	12th month	80.160±0.160 ^a	82.308±0.305 ^b	84.207±0.586 ^c	82.225±0.350
Normal Sperm (%)	11th month	93.622±0.417 ^a	93.700±0.380 ^b	94.128±0.441 ^c	93.817±0.413
	12th month	93.868±0.370 ^a	93.958±0.462 ^b	94.423±0.250 ^c	94.083±0.361
Live Sperm (%)	11th month	77.470±0.257 ^a	80.455±0.326 ^b	82.689±0.323 ^c	80.205±0.302
	12th month	80.269±0.153 ^a	82.842±0.373 ^b	85.355±0.895 ^c	82.822±0.474
Dead Sperm (%)	11th month	22.530±0.257 ^a	19.545±0.326 ^b	17.311±0.323 ^c	19.795±0.302
	12th month	19.731±0.153 ^a	17.158±0.373 ^b	14.645±0.895 ^c	17.178±0.474

Means with similar superscript in a row or column do not differ significantly among themselves

TABLE 4: ANOVA FOR AVERAGE VOLUME, COLOUR, MASS ACTIVITY, INDIVIDUAL MOTILITY, NORMAL **SEPRM, LIVE SPERM, DEAD SPERM COUNT OF ASSAM HILL GOAT OF DIFFERENT TREATMENT GROUPS**

Parameters	Source of Variation	df	SS	MS	F
Volume	Treatment	2	0.23	0.11	383.33**
	Month	1	0.04	0.04	133.33**
	Treatment x Month	2	0.00	0.00	0.00NS
	Error	30	0.01	0.00	
Color	Treatment	2	3.35	1.67	93.06**
	Month	1	1.73	1.73	96.11**
	Treatment x Month	2	0.32	0.16	8.89**
	Error	30	0.55	0.02	
Mass activity	Treatment	2	1.21	0.61	50.42**
	Month	1	0.90	0.90	75.99**
	Treatment x Month	2	0.13	0.07	5.42**

	Error	30	0.35	0.01	
Individual motility	Treatment	2	150.71	75.36	97.74**
	Month	1	77.03	77.03	99.91**
	Treatment x Month	2	7.55	3.77	4.89**
	Error	30	23.14	0.77	
Normal Sperm	Source of Variation	df	SS	MS	F
	Treatment	2	1.957	0.978	0.979
	Month	1	0.639	0.639	0.639
	Treatment x Month	2	0.004	0.002	0.002
	Error	30	27.749	0.925	
Live %	Treatment	2	159.62	79.81	64.36**
	Month	1	61.65	61.65	49.72**
	Treatment x Month	2	0.27	0.13	0.11
	Error	30	37.18	1.24	
Dead %	Treatment	2	159.62	79.81	64.41**
	Month	1	61.65	61.65	49.76**
	Treatment x Month	2	0.27	0.13	0.11
	Error	30	37.18	1.24	

**Highly significant ($P<0.01$)

4.CONCLUSION

Adding a polyherbal supplement (Shatavari root, Fenugreek, and Ajwain seed powder) at 1 g/kg and 2 g/kg of body weight, along with routine basal diet from weaning period, enhanced reproductive traits such as age at first heat, heat duration and age at sexual maturity in females as well as semen quality in males. However, there is a need for exploration of combinations with other medicinal herbs for optimal reproductive performance of the animals.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

No AI technologies have been used during preparation of the manuscript.

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