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Effect of area specific mineral mixture and antioxidants supplementation on semen evaluation of Barbari bucks

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Abstract
A study was conducted to assess the effect of area specific mineral mixture (ASMS) and antioxidant supplementation on serum profile of Barbari goats *viz.* Semen color (%), consistency (%) and volume (ml), Mass motility, Initial progressive motility (%), Sperm concentration, Sperm Abnormality, Percentage of live sperms, Host, Acrosome Integrity test (%), Two year old 18 Barbari bucks were selected for the study purpose consisting six bucks in each treatment group by simple random sampling. The bucks were housed in semi-intensive system of housing. Bucks were stall fed as well as pastured. The animals were divided into three group *viz*. control group, treatment 1 and treatment 2. Control group were fed with 300 gm of concentrate feed per head per day for 60 days. Treatment 1 bucks were given with 20g of area specific mineral mixture along with 300 gm of concentrate feed per head per day. Treatment 2 bucks were given with 20g of area specific mineral mixture, selenium 0.5 mg, vitamin- E 250 mg, vitamin -C 2 mg in addition with 300 gram concentrate feed per head per day for 60 days. Blood samples were collected after completion of the experiments in all treatment groups at 2 – 3 days interval and blood parameters were studied. The results of this study revealed that serum glucose levels differed significantly (P<0.05) between control and treatment groups.

Keywords: Barbari bucks, semen color, consistency and volume, mass motility, initial progressive motility, sperm concentration, sperm abnormality, live sperms

Introduction
Goats are the first domesticated ruminant animals. They are principally maintained by poorer section of the rural community providing them a source of livelihood. Therefore still the productivity of goats under the prevailing traditional production system is very low. Different breeds of goats produce a variety of products *viz*. milk, meat, fiber (mohair and pashmina) and manure. These products serve as a secure form of investment, a means of income and are used for various purposes including religious and ceremonial functions. The goat population has increased at a very fast rate making it an important species of animal for meat production. Still there is tremendous potential to be projected it as the 'Future Animal' for rural prosperity under the changing agro-geo-climatic conditions.

The main constraint in any livestock production including goat is the scarcity of good breeding stock, as good breeding stock is important to propagate the particular species. The achievement of high levels of fertility and prolificacy of goat depends not only upon the female members but also upon males [1]. In order to ensure the optimum reproductive performance of any group of breeding does in which natural service is used, a thorough examination and evaluation of the bucks for breeding soundness is important. The productive and reproductive performance of goat can further be enhanced by selective breeding of does with the bucks exhibiting desirable genetic characteristics [2]. The structural soundness, quality of semen, level of libido and plane of nutrition affect the male reproductive performance [3]. The fertility status of a male individual depends on series of factors which range from animal behavior and physical conditions, to features that are linked directly to the semen such as sperm motility, sperm morphology and biochemical constituents of the seminal plasma [4]. The reproductive performance of an individual animal can be altered through supplementation of micro minerals and antioxidants by nutrition, which can be assessed by semen estimation. Keeping these in a view, the present study was undertaken to assess the effect of area specific mineral mixture and antioxidant supplementation on semen evaluation of some semen parameters of Barbari bucks.

Reproductive performance is a function of both doe and buck fertility. Therefore, all aspects related to semen evaluation are important in management practices, especially for AI in a breeding program. Semen characteristics were found to be different among different breeds and among individuals of the same breed

Materials and Methods

The experiment was conducted at experimental shed complex on Central Institute for Research on Goat (C.I.R.G) Makhdoom, Mathura, Uttar Pradesh, India. Barbari bucks were used for the experiment. All the experimental bucks were marked with horn colour for easy identification. Subsequently Bucks were distributed randomly into one control group and two treatment groups, keeping initial body weight in consideration. The bucks were housed in semi-intensive system of housing. They were kept in well ventilated individual pens (3 x 5 Sq.ft) with mud floor and provided uniform housing.

Bucks had been pastured as well as stall fed. They were set on pasture both dawn and dusk during whole study period. Pasture used for grazing contained mainly Doob grass (*Cynodon dactylon*), Berseem (*Trifolium alexandrium*), Athua (*Chenopodium album*), Oats (*Avina sativa*) and leaves of Neem (*Azadirachta indica*), Poplar (*Populas alba*) and Ber (*Ziziphus indicus*). After feeding each group was turned out for natural grazing for 4 hrs. The experiment was continued for 60 days.

All experimental animals were pure Barbari goats. Animals were divided randomly into three groups (6 in each group) on basis of initial body weight (32 ±2 kgs) and kept almost same in every group. First group was kept as control and was fed with concentrated feed of 300 gram without mineral mixture or any kind of mineral supplementation, per head per day for 60 days. Second group (Treatment 1) was fed with 300 gram of concentrated feed fortified with 20g area specific mineral supplementation and Third group (Treatment 2) was fed with 300 gram of concentrated feed added with area specific mineral mixture 20g with selenium 0.5 mg, vitamin- E 250 mg, vitamin -C 2 mg, per head per day for 60 days. Vaccination and Deworming were carried out as per standard schedule and was carried out un-till the end of experiment. Necessary semen samples were collected from artificial vagina after two-three false mounts, using a goat as a teaser at 1, 3, 5, 8, 12, 15, 17, 19 and 21 days after the study period from each animal.

Estimation of semen sample in the glass tube was examined directly with naked eyes and graded as creamy, white or yellow colour, volume of the semen sample was recorded directly from graduated glass collecting tube in ml [1]. Consistency of semen sample was also recorded directly from graduated semen collecting tube as Thick and Thin [2]. Mass motility of spermatozoa in semen sample a drop of freshly collected semen was spread uniformly over a clean glass slide maintained at 30-35 ˚C and was examined under low power of the microscope (10X) [3]. Individual motility of spermatozoa, semen was diluted (1:100 dilution) in normal saline solution. One drop of the diluted semen was put on a clean dry slide and covered by cover slip. The slide was examined under high power (40X) of microscope [4]. Sperm concentration refers to the number of spermatozoa per ml of semen with the help of haemocyto meter in the same manner as RBC counts in blood [5]. Sperm abnormalities staining technique has been utilized for counting live and dead spermatozoa in semen smears. The dead sperms cell take eosin color, while are alive before staining do not take any color, and analysis through SPSS programme. One way ANOVA was employed to test the difference between various plasma parameters among treatment groups.

Results and Discussion

1. Semen Color

Average values for color and consistency (per cent) of semen samples from all Three Groups of barbari bucks on individual as well as overall basis are presented in Table 1 Results of semen color showed that Control group had 71.66 per cent samples creamy, 28.34 per cent yellowish in color and Treatment 1 had 80per cent samples creamy, 20per cent yellowish in color and Treatment 2 had 86.66per cent samples creamy, 13.34per cent yellowish in color. It has been reported that the semen samples with more spermatozoa concentration have cream to white color (Roberts, 1971) [1]. The yellow color is also normal and not related to any pathological change. Semen color is believed to be inherited (Ahmad and Noakes, 1996) [2].

Table 1: Semen colour (%)

Semen colour	N	Control	Treatment 1	Treatment 2
Percent creamy	180	71.66	75	86.66
percent yellowish	180	28.34	20	13.34

N-total no. of collection.

2. Semen Consistency (%)

Investigation on consistency of semen samples revealed that semen of barbari bucks Control group had 56.66per cent thick, 43.34per cent thin and Treatment 1 bucks had 66.66per cent thick, 33.34per cent thin and Treatment 2 bucks had 75 per cent thick, 25 per cent thin in consistency. Consistency of semen has direct relationship with sperm concentration of ejaculate. Samples having thick consistency showed maximum sperm concentration than samples with thin and watery consistency. Results of present investigation corroborate with (Roberts, 1971) [1] who reported that semen with spermatozoa concentration higher has to be thick whereas, samples having slightly lower sperm concentration have been reported to be thin and with spermatozoa concentration very less have been known to be watery.

Table 2: Semen Consistency (%)

Semen consistency	N	Control	Treatment 1	Treatment 222
Percent thick	180	56.66	66.66	75
percent thin	180	43.34	33.34	25

N- Total no. of collection.

3. Volume (ml)

Average values for volume (ml) of semen samples from all barbari bucks on individual as well as overall basis are presented in Table 3. The mean volume of semen obtained from barbari bucks. Volume of semen obtained from buck Treatment 2(0.73 ± 0.08 ml) was higher than that of Treatment 1 bucks (0.63 ± 0.08 ml) and Control Group (0.55 ± 0.07 ml). Overall mean volume of semen obtained in present study was higher than the Jamunapari buck (0.37 ml) as reported by Saxena and Tripathi (1980) [3], similarly Das *et al.* (2006) [17] reported semen volume 0.16 to 0.51 ml in Black Bengal buck. However it was less than semen volume of other documented breeds like Black Bengal (0.58 ml) and Barbari (0.92 ml)as mentioned in Banerjee (2005) [4]. Furstoss *et al.* (2009) [5] reported 0.48 ± 0.10 ml of semen volume from Alpine buck at 7 months of age which is slightly higher than the result of the present study. Variation in semen volume reported by different workers might be due to differences in genetics, reproductive health status, age, frequencyof

collection, nutrition, season and management (Nazir, 1988) [6].

Table 3: Mean ± SE of volume (ml) of Barbari bucks semen

Collections	Control	Group 1	Group 2
1	a 0.45±0.06	b 0.52±0.09	c 0.87±0.09
2	0.63±0.06	0.75±0.06	0.73±0.09
3	0.47±0.06	0.53±0.05	0.73±0.10
4	0.56±0.08	0.72±0.07	0.60±0.06
5	0.53±0.08	0.62±0.09	0.78±0.06
6	0.55±0.08	0.57±0.07	0.72±0.12
7	0.67±0.06	0.65±0.08	0.75±0.07
8	0.64±0.08	0.67±0.08	0.72±0.08
9	0.47±0.03	0.60±0.08	0.72±0.04
10	0.57±0.07	0.67±0.11	0.67±0.07

4. Mass motility

Average mass motility values (0 to 5 scale) of the spermatozoa for bucks. Mass motility of spermatozoa when graded on 0-5 scale was found in a range of 3.20 ± 0.20 to 3.80 ± 0.29 with an average value of (3.51± 0.08) which was at par with the score of Sannen breed (3.42) and higher than Nubian (3.19) as reported by Kamal *et al.* (2005) [7]. Similar mass motility (2.51 ± 0.28 to 3.99 ± 0.18) was reported by Ghalban*et al.* (2004) in Damascus buck. However in contrast to the results of present investigation Suyadi (2012) [8] reported lower value of mass activity (2.08 ± 0.16 to 2.53 ± 0.32) in Boer buck. The difference in motility in various reports could be due to variations in the judgment of motility, number of buck studied or difference of season at the time of studies.

Table 4: Mean ± SE of Mass motility of Barbari bucks

Collections	Control	Treatment 1	Treatment 2
1	3.42±0.20	4.25±0.36	4.00±0.22
2	3.58±0.20	3.67±0.17	3.50±0.18
3	3.42±0.20	3.50±0.13	4.17±0.21
4	a 3.50±0.18	b 3.42±0.20	c 4.42±0.24
5	a 3.58±0.15	b 3.75±0.21	c 3.83±0.11
6	3.42±0.15	3.42±0.20	4.42±0.30
7	3.67±0.25	3.58±0.15	3.92±0.15
8	3.25±0.17	3.67±0.17	3.92±0.30
9	3.58±0.15	3.75±0.28	4.00±0.13
10	3.50±0.26	3.58±0.15	4.00±0.26

5. Initial progressive motility (per cent)

The average mean values of individual motility (per cent) of spermatozoa for all Eighteen Barbari bucks on individual as well as overall basis are presented in Table 5. 60.75±2.68 per cent spermatozoa was found live in Control bucks and 63.61±2.60 per cent spermatozoa in Treatment 1 bucks and 66.99±3.89 per cent spermatozoa in Treatment 2 bucks respectively differences was noted in Initial progressive motility of sperm in among three group of bucks.

Table 5: Mean ± SE of Initial progressive motility of Barbari bucks.

Collections	Control	Treatment 1	Treatment 2
1	64.17±3.74	62.50±2.51	69.00±4.16
2	56.33±1.91	57.33±1.58	63.33±2.08
3	60.67±4.55	66.00±2.97	65.00±3.11
4	61.83±2.29	71.67±3.91	61.33±3.17
5	a 61.17±2.30	ac 62.00±2.00	c 73.00±2.58
6	62.50±3.66	63.50±3.31	68.00±4.09
7	62.83±1.99	63.00±2.25	62.50±2.86
8	61.00±3.27	66.00±2.38	67.33±2.89
9	a 56.50±1.52	ac 65.17±3.32	c 73.17±3.42
10	60.50±1.65	59.00±1.77	67.33±2.78

6. Sperm concentration

The mean value of sperm concentration (m/ml) was higher for Treatment 2 bucks (401.17 ± 21.30 x 10^6/ml)compared to that Treatment 1 bucks (354.50 ± 31.98 x 10^6/ml) and Control Group (243.84 ± 15.93 x 10^6/ml)bucks on individual as well as overall basis are presented in Table 6.Studies conducted by Apu (2007) and Afroz (2005) [9] also revealed that the average sperm concentration of cross bred buck semen was 2.43 ± 52.81 to 2.85 ± 90.12 10^9/ml which is in accordance with the present results. However higher values than present study were recorded in Malabari (3.53 ± 0.176 x $10^{9/}$ml) and lower in Black Bengal (2.289 x 10^9/ml) buck (Singh *et al.*, 1985) [10]. Results of present study show agreement with Kabiraj *et al.* (2011) [11] who observed sperm concentration (2.07 ± 0.12 x 10^9/ml) in buck of age (0.5 to 1.0 year), (3.12 ± 0.14 x $10^{9/}$ml) in buck of age (1.5 to 3 years). The difference in results on concentration may be due to variation in age, breed, collection frequency, feeding regime and climatic condition (Leon *et al.*, 1991) [12]. Roca *et al.* (1992) [13].

Table 6: Mean ± SE of Sperm Concentration (x 10^6 /ml) of Barbari bucks.

Collections	Control	Treatment 1	Treatment 2
1	a 233.33±13.08	b 360.00±24.77	c 418.33±24.14
2	a 235.00±12.85	b 368.33±23.01	c 396.67±09.19
3	a 265.00±20.78	ac 333.33±41.45	c 391.67±26.26
4	a 255.00±16.07	ac 348.33±43.77	c 426.67±28.48
5	a 255.00±23.20	ac 325.00±33.54	c 423.33±37.03
6	a 213.33±13.82	b 373.33±40.47	c 395.00±19.79
7	a 235.00±16.88	b 370.00±22.66	c 386.67±18.20
8	a 250.00±08.94	b 358.33±22.72	c 441.67±19.56
9	a 246.67±17.26	b 366.67±38.53	c 370.00±16.12
10	a 250.00±16.33	ac 341.67±28.92	c 361.67±14.24

7. Sperm abnormalities

Sperm abnormalities (per cent) have been classified on the basis of their origin. Sperm defects which occur during spermatogenesis are considered as sperm abnormalities. Semen samples from sixteen bucks were collected and pooled. Then three replicate slides were counted from each slide or overall were analyzed for assessment of sperm abnormalities. Sperm were examined sperm abnormalities. Results on individual as well as overall basis are presented in Table 7.

Table 7: Mean ± SE of Sperm Abnormality of Barbari bucks

Collections	Control	Treatment 1	Treatment 2
1	1.67±0.67	0.83±0.31	0.67±0.33
2	1.33±0.61	1.00±0.37	0.67±0.21
3	1.17±0.48	0.83±0.31	0.67±0.33
4	1.67±0.61	1.17±0.48	0.33±0.21
5	1.33±0.67	1.33±0.42	1.17±0.31
6	1.17±0.40	0.83±0.31	0.67±0.21
7	1.50±0.62	1.00±0.37	0.50±0.34
8	1.50±0.62	0.67±0.33	0.83±0.31
9	1.67±0.42	1.17±0.17	0.67±0.21
10	1.83±0.65	0.67±0.33	1.17±0.17

8. Percentage of live sperms

The average mean values of sperm livability (per cent) for all eighteen Barbari bucks on individual as well as overall basis are depicted in Table 8. 56.67±3.40per cent spermatozoa was found live in Control bucks and 60.08±3.55 per cent spermatozoa in Treatment 1 bucks and 70.60±4.01 per cent spermatozoa in Treatment 2 bucks respectively. Differences

was noted in livability of sperm in among three group of bucks. The results obtained in present study are similar to those obtained by Raza *et al*. (2006) [14] who reported that number of viable spermatozoa decreased from 76.66 per cent to 19.22 per cent after cold shock treatment at 0 °C in Beetal buck. Perez-Pe*et al.* (2001) [15] noted higher value of live spermatozoa at 0 °C (24.6 ± 2.1 per cent) in rams compared to present study, which might be due to species difference. Difference in livability of sperm may be due to sudden fall in temperature and it is noted that goat spermatozoa are very sensitive to cold shock and can't be cooled to 5 °C in the absence of egg yolk (Salamon and Maxwell, 1995) [16].

Table 8: Mean ± SE of Sperm Live % of Barbari bucks.

Collections	Control	Treatment 1	Treatment 2
1	59.75±5.11	62.00±5.16	70.00±4.65
2	a 59.00±2.73	b 59.50±2.54	c 76.50±4.57
3	a 58.70±6.52	b 59.83±5.84	c 76.00±4.19
4	a 56.17±3.37	b 56.20±3.20	c 73.00±4.43
5	58.07±2.06	56.75±3.55	64.33±4.13
6	a 51.50±2.20	ac 66.33±5.21	c 71.50±3.86
7	61.00±3.53	66.52±3.93	66.40±1.95
8	56.83±2.89	62.83±3.27	71.50±3.17
9	a 53.33±3.33	ac 55.83±1.56	c 70.50±5.59
10	52.33±2.23	55.00±1.24	66.25±3.38

Conclusion

A statistically significant difference is noted in the semen colour, consistency, semen volume, mass motility, Initial progressive motility, sperm concentration, Livability, sperm abnormalities s level between area specific mineral mixture and antioxidants supplement group than the control group.

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