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RESEARCH ARTICLE

Fenugreek seed supplementation as galactagogue in Patanwadi sheep and its effect on milk yield, milk quality and feeding behaviour

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Abstract: Phytogenic feed additives have become an important component in animal nutrition. Fenugreek seeds, known for its medicinal properties, are also classified as potential galactogogues. Therefore, the present research investigated the potential of fenugreek seed supplementation on milk production in Patanwadi ewes. Twenty-four lactating ewes with similar milk yield were randomly allocated to 3 equal groups. Group I (Control) was maintained on a basal diet without supplementation of fenugreek seeds, whereas Group II (Treatment 1) and Group III (Treatment 2) were supplemented with 2.5 and 5% fenugreek seeds of the basal diet, respectively. Feeding behaviour of ewes was recorded on a weekly basis for the initial five weeks, which included sniff bout, paw bout, snuff bout and feed intake time. Milk yield was recorded at weekly intervals for 13 weeks, while milk composition and blood samples were analyzed during the 1st, 5th, 9th and 13th week. Fenugreek seed supplementation exhibited a positive effect on milk production in lactating ewes. Milk yield was higher ($p < 0.05$) in T_2 compared to the control. The milk composition was also influenced, with the mean fat percentage significantly ($p < 0.05$) lower and SNF significantly ($p < 0.05$) higher in treatment groups compared to the control. Feeding behaviours (paw bout, sniff bout, snuff bout) were comparable in treatment but were significantly different from the control. A higher economic return was also observed in treatment groups compared to the control.

Keywords: Fenugreek seed, Feeding behaviour, Milk composition, Prolactin, Sheep milk

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Introduction

Over the last few years, phytogenic feed additives have become popular due to their potential as sustainable substitutes in animal nutrition, significantly because of drug resistance issues and the environmental effects of synthetic supplements (Seal et al. 2013). Of these, fenugreek (*Trigonella foenum-graecum)* is one of the most ancient medicinal plants, traditionally esteemed since Hippocrates' time and ancient Egypt (Jensen, 1992). A yearly herb belonging to the *Papilionaceae-Leguminosae* family, is largely planted in India, the Middle East, Northern Africa, and Southern Europe (Fotopoulos, 2002) and found to be effective in improving lactation performance in various dairy animal models, with reported increases in milk yield and quality (Al-Shaikh et al. 1999; Shah and Mir, 2004; Samia et al. 2012 Akbag et al. 2022; Zeng et al. 2024). Sheep milk is rich in fat and SNF content and suitable to prepare various dairy products. The composition of the milk and milk flavor are changed by the dietary factors. Raw fenugreek seeds possess a bitter taste because of the presence of saponins, which reduces their acceptability in food products (Patel et al. 2016). Nevertheless, in spite of a long history of use in traditional medicine, fenugreek's galactogogue activity on sheep milk in Indian semi-arid conditions has not been explored. The purpose of this research was to assess the influence of dietary supplementation of fenugreek seeds on milk yield, lactation performance, and weight gain in Patanwadi ewes. The research further sought to explore how fenugreek contributes to increased milk production by determining the levels of prolactin hormone. The feeding patterns of the sheep were also followed to ascertain their acceptance of the fenugreek seed, taking into account its strong flavour.

Materials and Methods

Study area

The current research was carried out at Sector-15 of the Indian Council of Agricultural Research – Central Sheep and Wool Research Institute (ICAR-CSWRI), Avikanagar, Malpura, Rajasthan, India. The campus is located at 26° 122 52.23 N (26.2145° N) latitude and 75° 452 24.843 E (75.7569° E) longitude

at 320 meters above mean sea level. The Experiment was carried out from the month of January to March 2022 for 90 days.

Animal selection

For this research work, twenty-four (24) Patanwadi ewes were selected and divided into three groups based on similar parity and milk yield of previous parity to maintain uniformity.

Feeding experimental design

All the animals were allowed free access to graze in the fenced pasture during daytime (8.00- 16.00 h), along with drinking water during midday at 12.00 h. In addition to grazing, each ewe was supplemented with 400 g concentrate mixture (Table 1) as a basal diet in mash form. The proximate analysis of concentrate and fenugreek seed was determined and presented in Table 2. For the feeding trial, Group I (control) was maintained on a basal diet without fenugreek supplementation, whereas Group II (Treatment 1) and Group III (Treatment 2) were supplemented with 2.5% and with 5% fenugreek seeds of basal diet, respectively. The whole duration of the experiment was 90 days. The animals were maintained under homogeneous managemental conditions and were kept in separate pens during concentrate feeding to prevent competition with *ad libitum* access to fresh water.

Sample collection and analysis

Milk yield was recorded at weekly intervals using the weigh–suckle–weigh technique, wherein lambs were weighed before and after suckling twice daily (morning and evening). Milk samples were also collected after 1 minute of lamb suckling during early to mid-lactation for their compositional analysis such as pH, fat, solids-not-fat (SNF), protein, lactose, salt and density during the 1st, 5th, 9th, and 13th weeks of lactation. The collected milk samples were transferred within 1 h of collection to the Livestock Products Technology Laboratory, ICAR-CSWRI, in a cool box for compositional analysis using an automatic milk analyzer (Lactoscan Combo, Milkotronic Ltd., Bulgaria). Around 3-4 ml venous blood was collected from the experimental ewes at the 1st, 5th, 9th, and 13th weeks of lactation, and the separated serum was analyzed for prolactin concentration using radioimmunoassay (RIA). Apart from milk parameters, the feeding behaviour of experimental ewes was monitored weekly during the first month of the experiment. The observed traits were sniffing, pawing, snorting and total feeding time.

Statistical analysis

The collected data were analyzed using SPSS software (SPSS, 27.0). One-way analysis of variance (ANOVA) was employed to compare the means of the groups. Repeated measures ANOVA were employed for data collected at several time points. Post hoc multiple comparisons were made using Duncan's multiple range test.

Results and Discussion

Effect on body weight of ewe

The mean body weight of ewes at lambing and 13 weeks of lactation is presented in Table 3. The initial body weight was comparable among the groups, with values of 41.62 kg in the control, 41.04 kg in T_1 and 40.85 kg in T_2. At the end of the experiment, the body weight was 42.13 kg, 41.23 kg, and 41.62 kg, in control, T_1 and T_2, respectively. All three groups showed a marginal increase in body weight, although these differences were not statistically significant ($p > 0.05$). The results indicate that fenugreek supplementation had no adverse effects on body weight or overall performance of lactating ewes. A modest positive effect on body weight with fenugreek supplementation has been reported by Samia et al. (2012) in Sudanese desert lactating sheep and Akbag et al. (2022) in lactating goats.

Effect on milk yield

The weekly milk yield of Patanwadi sheep is presented in Table 4; Fig 1. The milk yield increased up to the 5th week in the control and treatment groups, followed by a gradual decline till the 13th week, reflecting the characteristic pattern of a normal lactation curve in ruminants (Hasin et al. 2021). Fenugreek seed supplementation had no significant effect ($p > 0.05$) on weekly milk yield except for the 5th and 10th week; however, higher values were reported in the treatment groups. The overall mean milk yield was observed to be significantly higher in the treatment groups (711.06, 687.50 grams for T_2 and T_1, respectively) than in the control (614.23 grams). Similarly, Samia et al. (2012) reported that milk yield was enhanced in Sudanese desert sheep by supplementation of fenugreek seed at the rate of 2.5 and 5.0 gram/kg body weight/day over a period of 7 weeks. The daily milk yield was reported to be higher in Hamdani ewes with the addition of crushed fenugreek seed as a feed additive to the basal ration at the levels of 0.6 and 1.2g/kg body weight (Al-Sherwany, 2015). The results of higher milk yield through fenugreek seed supplement were consistent with the findings of Çayiroðlu et al. (2023) in goats; Hasin et al. (2021) in goats of Kashmir valley; Mahgoub et al. (2015) in Egyptian buffaloes and Kirar et al. (2020) in Murrah buffaloes. This positive effect is attributed to the galactagogue activity of fenugreek seed due to

Table 1: Composition of the concentrate offering to ewes in the experiment

Feed ingredients	Percentage (%)
Maize Grain	40
Barley Grain	24
De-oiled Groundnut Cake	12
De-oiled Mustard Cake	10
Sesamum Cake	11
Mineral Mixture	2
Common Salt	1

the presence of diosgenin, which stimulates prolactin hormone secretion and increases milk production (Akbag et al. 2022). Abo El-Nor (1999) also reported that feeding of fenugreek seeds in the diet of lactating buffaloes increased prolactin hormone level, which is one of the key hormones regulating milk synthesis and secretion. These findings indicate that dietary inclusion of fenugreek exerted a positive effect on the milk yield performance in lactating ruminants.

Effect on serum Prolactin

Prolactin concentrations were measured at the onset of the experiment and thereafter at the 5th, 9th and 13th weeks of lactation (Fig 2). At the initial stage, prolactin levels were comparable across all the groups, reflecting no baseline differences. As lactation progressed, prolactin concentrations dropped gradually in all the groups within the normal physiological range. However,

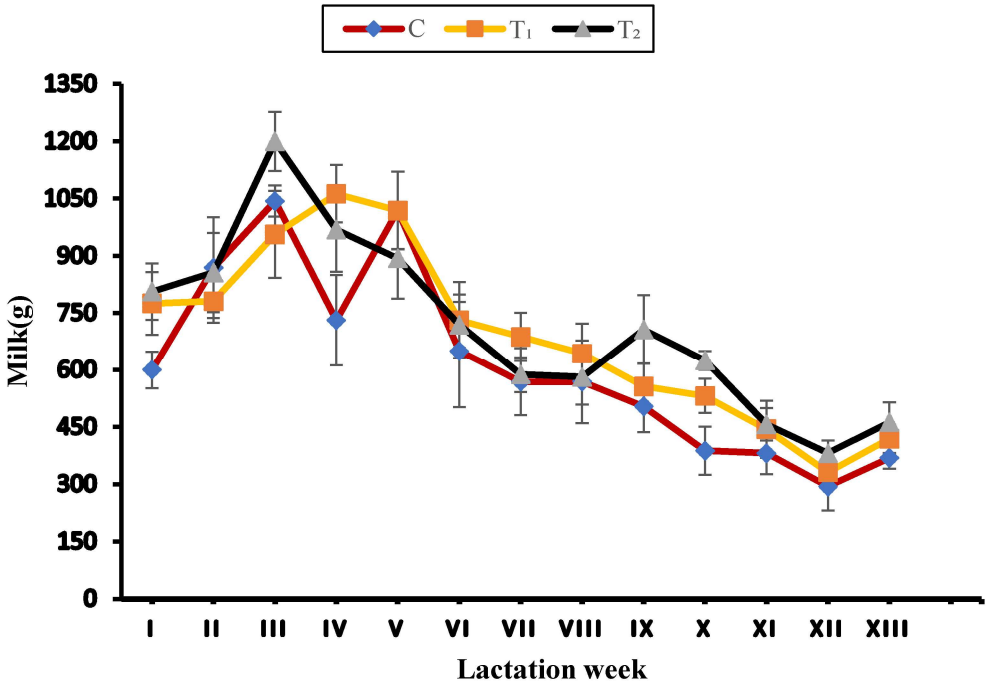

Fig. 1. Weekly mean milk production (g) in different treatments

Table 2: Proximate analysis of the concentrate feed and fenugreek seed

Proximate Composition	Concentrate (%)	Fenugreek Seed (%)
Dry matter	95.90	96.34
Organic matter	92.33	95.87
Crude protein	18.44	27.75
Total Ash	7.67	4.13
Ether extract	1.61	6.94
Neutral detergent fiber	37.52	-
Acid detergent fiber	10.90	-
Lignin	3.34	-
Hemicellulose	26.62	-
Cellulose	7.55	-

Table 3: The effect of fenugreek supplementation on the body weight of ewes

Group	Weight at lambing (kg)	Weight at 13th week of lactation (kg)
C (n=8)	41.62 ± 1.06	42.13 ± 0.87
T1 (n=8)	41.04 ± 1.13	41.23 ± 0.89
T2(n=8)	40.85 ± 1.95	41.62 ± 2.05

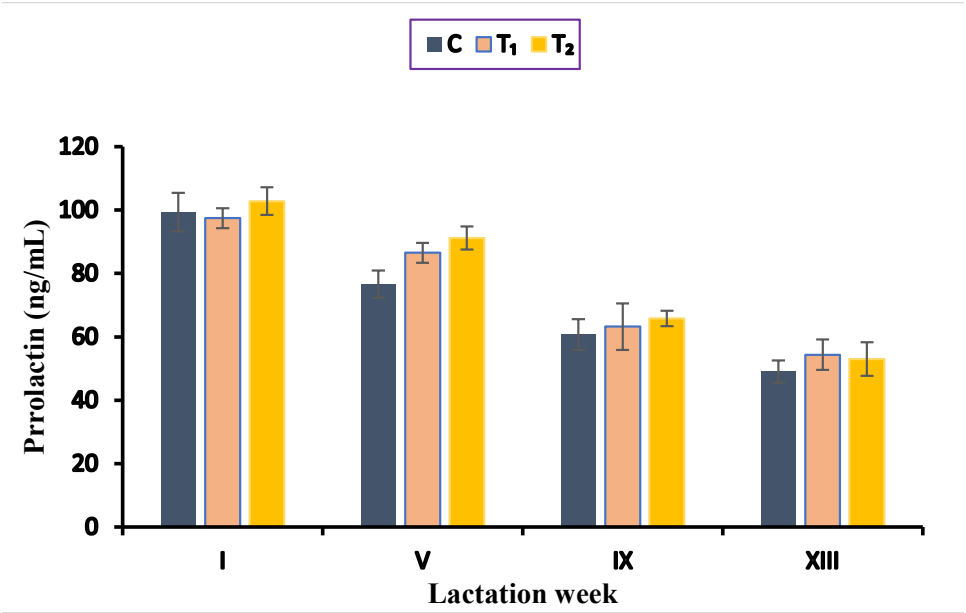

Fig. 2. Prolactin hormone concentration at different weeks of lactation

Table 4: The effect of fenugreek supplementation on the weekly milk yield (gram) in Patanwadi sheep

Time interval	Milk Yield (gram)		
	Control (n=8)	T_1 (n=8)	T_2(n=8)
1st week	600.00 ± 48.18	775.00 ± 82.38	806.25 ± 74.06
2nd week	868.75 ± 132.27	781.25 ± 56.65	856.25 ± 103.70
3rd week	1043.75 ± 40.57	956.25 ± 113.95	1200.00 ± 77.34
4th week	731.25 ± 118.75	1062.50 ± 75.45	968.75 ± 110.98
5th week	1018.75^{a} ± 101.30	1018.75^{ab} ± 101.74	893.75^{b} ± 106.67
6th week	650.00 ± 148.20	731.25 ± 99.97	718.75 ± 60.46
7th week	568.75 ± 88.10	687.50 ± 63.21	587.50 ± 46.05
8th week	568.75 ± 108.54	643.75 ± 78.74	581.25 ± 72.54
9th week	503.75 ± 67.85	556.25 ± 59.34	706.25 ± 89.86
10th week	387.50^{b} ± 63.21	531.25^{a} ± 45.26	625.00^{a} ± 25.00
11th week	381.25 ± 55.85	443.75 ± 74.66	456.25 ± 42.72
12th week	293.75 ± 62.28	331.25 ± 40.02	381.25 ± 32.65
13th week	368.75 ± 36.52	418.75 ± 28.25	462.50 ± 51.54
Mean milk	614.23^{b} ± 32.83	687.50^{ab} ± 29.34	711.06^{a} ± 29.21

Different superscripts (a, b) indicate a significant ($p < 0.05$) difference between the groups.

among the groups, prolactin concentrations were higher in T_2, followed by T_1, compared to the control. At the 5th week, prolactin concentration was significantly ($P<0.05$) higher in T_2 (91.17 ng/dl) compared to the control (76.64 ng/dl), while T_1 exhibited a numerically higher value without statistical significance. The elevated prolactin concentrations in the treatment groups, especially T_2, may be attributed to the galactagogue property of fenugreek, which is known to stimulate prolactin secretion and thereby promote lactation performance (Akbag et al. 2022). The findings are in agreement with Samia et al. (2012), who reported elevated serum prolactin levels in Sudanese desert sheep, and Al-Janabi (2012), who observed a similar effect in lactating goats. Likewise, Çayiroðlu et al. (2023) observed increased prolactin concentrations in Akkeci goats supplemented with 50 g of fenugreek.

Effect on milk composition

The chemical composition of milk at the 1st, 5th, 9th, and 13th week of lactation is presented in Table 5. The milk pH, a critical indicator of udder health and mastitis incidence, remained within the normal physiological range with the mean value of 7.16 to 7.26 in all groups during the experimental period. The addition of fenugreek seeds in the diet has no significant adverse effect on milk pH; however, a gradual increase in pH values was noticed throughout the lactation. The present findings are in agreement with the trends reported by Rathore et al. (2021) in lactating cows up to 6 weeks. The animals in all the groups showed an increase in fat percentage with the advancement of lactation, due to a progressive decline in milk yield with time. However, fenugreek supplementation resulted in a significant ($p < 0.05$) decline in

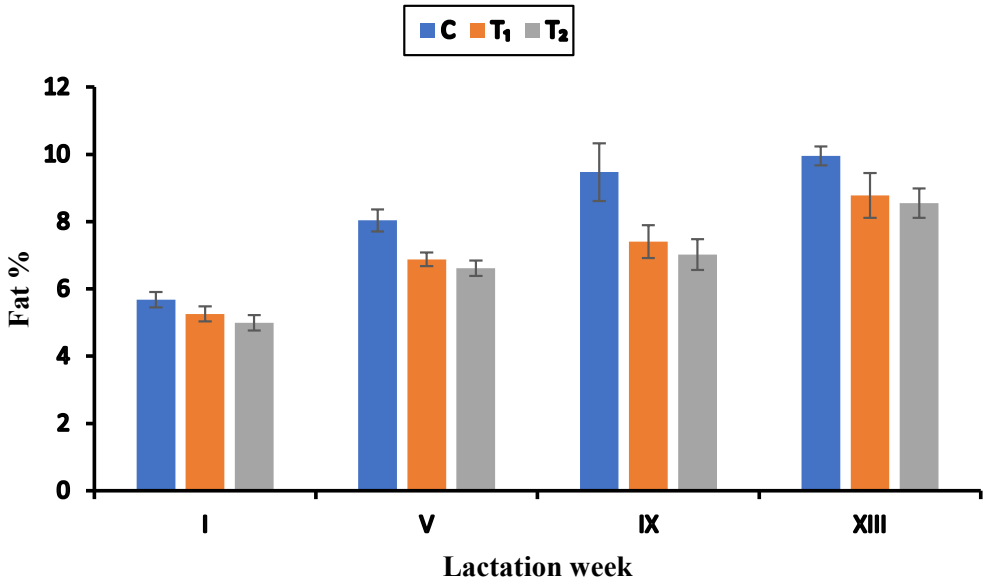

Fig. 3. Fat percent at different weeks of lactation

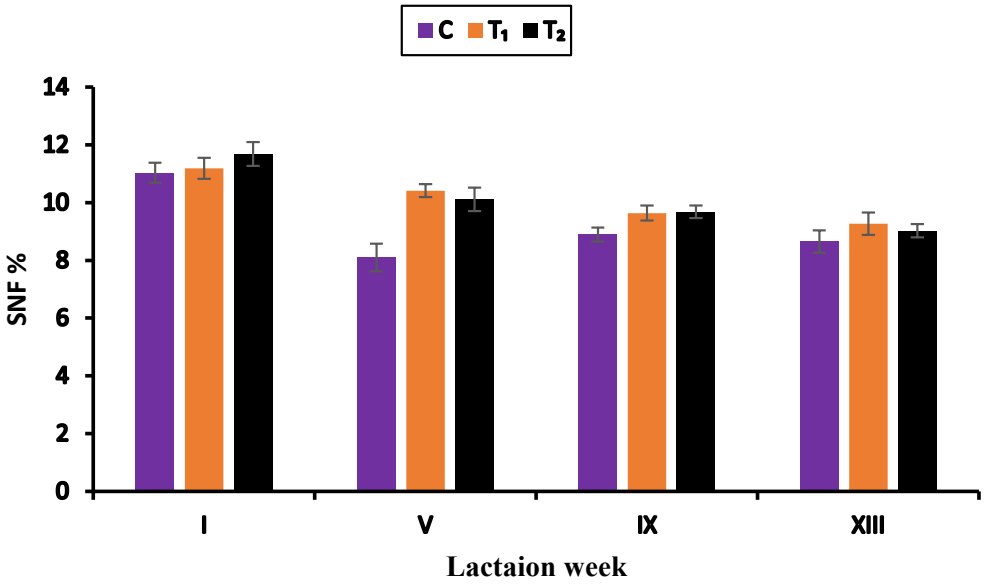

Fig. 4. SNF percent at different weeks of lactation

mean fat percentage in the treatment groups (7.08±0.31 and 6.80±0.28 in T_1 and T_2 groups, respectively) as compared to the control (8.29 ±0.38). The relatively lower fat content in the supplemented groups could be associated with higher milk yield, as there exists a negative correlation between milk yield and milk fat percent. These results are in agreement with the findings of Al-Sherwany (2015), who reported that adding fenugreek seed to the diet of Hamdani ewes reduced the milk fat percent. The similar findings were also observed by Choubey et al. (2018) in Surti buffaloes with supplementation of 1.5 % fenugreek seed in the ration. The reduction in milk fat might be due to the presence of saponins in fenugreek seeds, which increases the ruminal concentration of propionic acid and reduces the concentration of acetic acid, leading to milk fat depression (Hart et al. 2008). The solid-not-fat (SNF) percentage was higher during the initial stage in all groups but decreased sharply as lactation progressed. However, the average SNF content was significantly ($p < 0.05$) higher in the fenugreek-supplemented group (10.13%) compared to the control (9.17%), which suggested that fenugreek contributed positively to the non-fat solids portion of milk. Kirar et al. (2020) reported that supplementation of 50 g of soaked fenugreek seeds increased the SNF content in Murrah buffaloes. Likewise, results were also obtained by Akbag et al. (2022) in Turkish Saanen dairy goats with supplementation of 100 g fenugreek seed/ animal in total mixed ration (TMR). The milk protein content also followed a similar trend like SNF. The mean

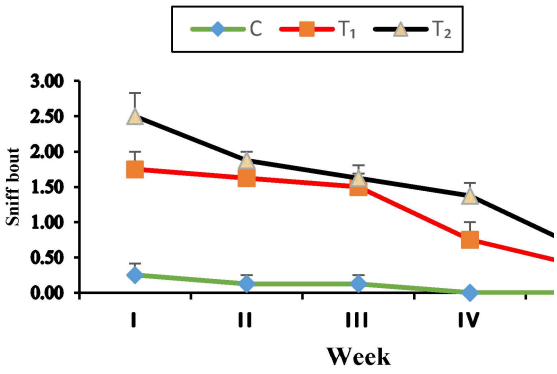

Fig. 5a. Feed sniffing behavior in sheep

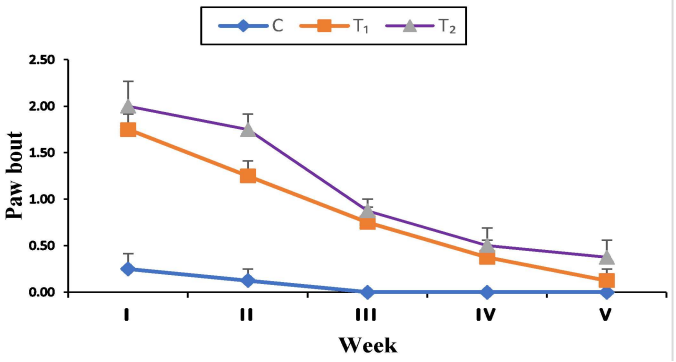

Fig. 5b. Pawing behavior

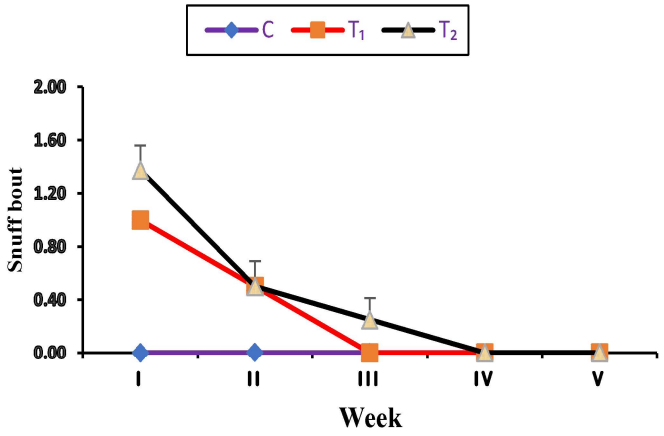

Fig. 5c. Snuffing behviour

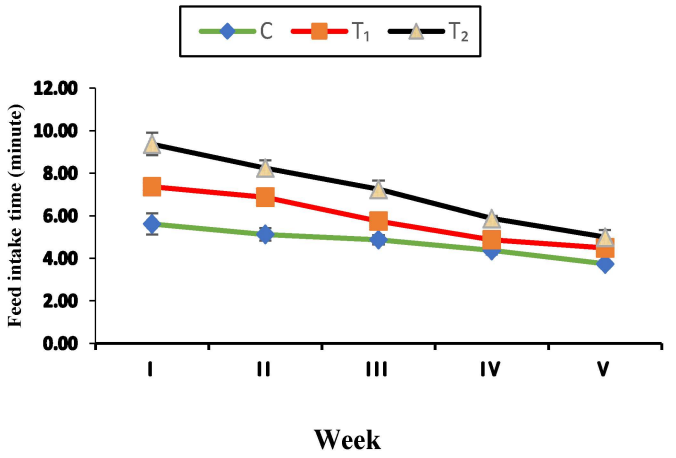

Fig. 5d. Feed intake time in different groups

protein content was significantly (P<0.05) higher in treatment groups (3.68%) than the control (3.31%). Hasin et al. (2021) reported comparable values for milk protein content in Kashmir valley goats with 30 g daily fenugreek supplementation. No significant difference was observed in lactose percentage among the groups. These results are in agreement with findings of Akbag et al. (2022) in Turkish Saanen dairy goats and Choubey et al. (2018) in Surti buffaloes. Salt content of milk was not affected by fenugreek supplementation. Density of milk decreased with lactation period, but it was significantly ($p < 0.05$) higher in treatment groups compared to control, and these results are in accordance with the findings of Choubey et al. (2018) in Surti buffaloes.

Feeding behavior

Feeding behaviour (sniffing, pawing, snout-bouts, and feed intake time), was observed to assess their response and acclimatization to fenugreek supplementation (Figures 5a–d). Sniffing bout frequency was significantly (P<0.05) higher in fenugreek-fed groups than in the control, with T_2 showing a numerically higher response than T_1; however, sniffing behaviour decreased progressively in all groups, disappeared by the fourth week in the control, while persisted in treatments. Similarly, paw-bout behaviour followed a similar pattern to sniffing bout, initially higher in treatment groups—especially T_2 compared to the control, but declined steadily with time and from the third week onwards it disappeared in the control diet while mildly persisted in treatment up to 5th week indicating gradual acceptance of the supplemented feed. Likewise, snout-bouts were absent in the control but appeared in both treatment groups during the initial weeks, disappeared by the 3rd week in T_1 and 4th week in T_2, showing early strong non-acceptance but gradually acceptance of fenugreek seeds. Feeding time was 66.6 and 31.1 percent longer in T_2 and T_1 groups than that in the control during the early phase, reflecting reluctance and slower ingestion due to the strong flavour of fenugreek, but it gradually decreased across all groups as adaptation improved. By the 5th week, feeding time remained highest in T_2, followed by T_1 and control, suggesting that although the ewes were acclimatized to fenugreek, but its distinct aroma and taste continued, which prolonged the intake time in the treatments compared to the control.

Table 5: The effect of fenugreek supplementation on the milk composition in Patanwadi sheep

Group (n=8)	Milk composition				
	1st week	5th week	9th week	13th week	Mean
			pH		
C	$6.68^{b} \pm 0.03$	$8.40^{aA} \pm 0.75$	$6.88^{bA} \pm 0.17$	$7.09^{b} \pm 0.46$	7.26 ± 0.24
T_1	7.37 ± 0.39	$6.93^{B} \pm 0.24$	$7.41^{B} \pm 0.24$	7.50 ± 0.49	7.30 ± 0.17
T_2	6.74 ± 0.24	$7.18^{AB} \pm 0.11$	$7.06^{B} \pm 0.17$	7.68 ± 0.52	7.16 ± 0.16
			Fat%		
C	$5.68^{c} \pm 0.23$	$8.04^{bA} \pm 0.33$	$9.47^{aA} \pm 0.86$	$9.95^{a} \pm 0.28$	$8.29^{A} \pm 0.38$
T_1	$5.26^{c} \pm 0.22$	$6.88^{bB} \pm 0.20$	$7.40^{bB} \pm 0.49$	$8.78^{a} \pm 0.67$	$7.08^{B} \pm 0.31$
T_2	$4.99^{c} \pm 0.23$	$6.62^{bB} \pm 0.23$	$7.02^{bB} \pm 0.46$	$8.55^{a} \pm 0.44$	$6.80^{B} \pm 0.28$
			SNF%		
C	$11.03^{a} \pm 0.35$	$8.10^{bB} \pm 0.48$	$8.89^{bB} \pm 0.24$	$8.65^{b} \pm 0.39$	$9.17^{B} \pm 0.27$
T_1	$11.19^{a} \pm 0.36$	$10.42^{abA} \pm 0.23$	$9.64^{bcA} \pm 0.26$	$9.27^{c} \pm 0.38$	$10.13^{A} \pm 0.20$
T_2	$11.69^{a} \pm 0.41$	$10.12^{bA} \pm 0.40$	$9.69^{bcA} \pm 0.22$	$9.03^{c} \pm 0.23$	$10.13^{A} \pm 0.23$
			Protein %		
C	$4.04^{a} \pm 0.13$	$2.92^{bB} \pm 0.17$	$3.20^{bB} \pm 0.09$	$3.10^{b} \pm 0.15$	$3.31^{B} \pm 0.10$
T_1	$4.09^{a} \pm 0.14$	$3.80^{abA} \pm 0.09$	$3.49^{bcA} \pm 0.10$	$3.34^{c} \pm 0.15$	$3.68^{A} \pm 0.08$
T_2	$4.28^{a} \pm 0.15$	$3.68^{bA} \pm 0.15$	$3.51^{bcA} \pm 0.08$	$3.26^{c} \pm 0.09$	$3.68^{A} \pm 0.09$
			Lactose%		
C	$6.08^{a} \pm 0.19$	$4.47^{bB} \pm 0.26$	$4.96^{b} \pm 0.15$	$4.78^{b} \pm 0.22$	$5.07^{B} \pm 0.15$
T_1	$6.16^{a} \pm 0.20$	$5.74^{abA} \pm 0.13$	$5.33^{bc} \pm 0.14$	$5.12^{c} \pm 0.21$	$5.59^{A} \pm 0.11$
T_2	$6.40^{a} \pm 0.23$	$5.58^{bA} \pm 0.22$	$5.34^{bc} \pm 0.12$	$4.98^{c} \pm 0.12$	$5.57^{A} \pm 0.13$
			Salt %		
C	$0.97^{a} \pm 0.05$	$0.66^{bB} \pm 0.04$	$0.72^{b} \pm 0.02$	$0.70^{b} \pm 0.03$	0.76 ± 0.03
T_1	$0.95^{a} \pm 0.05$	$0.85^{bA} \pm 0.02$	$0.78^{bc} \pm 0.02$	$0.75^{c} \pm 0.03$	0.83 ± 0.02
T_2	$0.96^{a} \pm 0.04$	$0.82^{bA} \pm 0.03$	$0.79^{bc} \pm 0.02$	$0.72^{c} \pm 0.01$	0.82 ± 0.02
			Density		
C	$35.53^{a} \pm 1.25$	$23.76^{bB} \pm 1.71$	$25.52^{bB} \pm 1.09$	$24.10^{b} \pm 1.68$	$27.22^{B} \pm 1.11$
T_1	$36.71^{a} \pm 1.19$	$32.74^{bA} \pm 0.77$	$30.10^{bcA} \pm 1.23$	$27.53^{c} \pm 1.89$	$31.77^{A} \pm 0.88$
T_2	$38.85^{a} \pm 1.27$	$32.13^{bA} \pm 1.40$	$30.62^{bA} \pm 0.93$	$26.84^{c} \pm 1.20$	$32.11^{A} \pm 0.97$

Different superscripts (a, b, c) indicate a significant ($p < 0.05$) difference within the groups, while (A, B) indicate a significant ($p < 0.05$) difference between the groups.

Table 6: Economic efficiency of lactating ewes fed the experimental rations

Particulars	Groups		
	Control	T_1	T_2
Total milk production (90 days) (liter/ewe)	55.28	61.87	64
Extra milk production (liter/ewe)	-	6.59	8.72
Cost of extra milk produced @ 50 Rs/liter	-	329.5	436
Fenugreek seed (90 days) (kg/animal)	-	0.9	1.8
Cost of fenugreek seed feeding @ 45 Rs/kg		40.5	81
Extra Income/ewe (Rs)	-	289	355
B:C ratio	-	8.14 : 1	5.38 : 1

Economics of fenugreek seed

The results of the study revealed that supplementation of fenugreek seed improved milk yield in lactating ewes, thereby enhancing the economic return from milk production (Table 6). It was observed that supplementation of fenugreek seed @ 2.5% and 5% resulted in additional income of Rs. 289 and 355, respectively, over the control. However, based on the benefit–cost (B:C) ratio, T_1 (8.14:1) provided a higher economic return compared to T_2 (5.38:1).

Conclusions

The results of the present study revealed that adding fenugreek seeds to the diet of ewes resulted in increased milk yield, with maximum production observed at 5% inclusion level. Prolactin

concentrations were also higher in fenugreek-fed groups, indicating that fenugreek might have a stimulatory role in lactation. Milk composition improved with fenugreek supplementation, showing positive influence on SNF and protein content and reduction in fat content. Body weight of the ewes was not adversely affected, indicating that fenugreek seed can be safely incorporated in the diet of Patanwadi ewes without compromising health. Although, initial feed acceptability was low due to the bitter taste of fenugreek; however, after one month of feeding, the ewes were gradually adapted to fenugreek supplementation. Economic assessment indicated that inclusion of 2.5% fenugreek seed was more cost-effective than 5%. These findings highlighted fenugreek seed supplementation as a potentially safe, economically viable and sustainable approach to increase productivity in lactating ewes.

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Conflict of Interest

All authors declare that they have no conflict of interest that could have appeared to influence the work reported in the paper.

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