EFFECT OF FENUGREEK (*METHI*) SUPPLEMENTATION ON MILK PRODUCTION, MILK COMPOSITION AND HAEMATO-BIOCHEMICAL PARAMETERS IN MURRAH BUFFALOES

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ABSTRACT

The envisaged research was undertaken to study the effect of Fenugreek (*Methi*) supplementation on milk production, milk composition and haemato-biochemical parameters in Murrah buffalo. In this study, eighteen lactating Murrah buffaloes were randomly divided into three treatment groups having six animals in each on the basis of milk yield, body weight and parity. Basal roughage-concentrate mixture diets as per ICAR (2013) feeding standards were provided to the buffaloes in the control group (T_1). The experimental treatment groups included basal ration (control) supplemented with crushed fenugreek seeds @150 g per animal per day (T_2) and basal ration (control) supplemented with crushed fenugreek seeds @250 g per animal per day (T_3). The experimental animals were housed under loose housing system following standard managemental practices and biosecurity measures. The experimental period was from 10th day postpartum to completion of 6th month of lactation of all the experimental animals. The results of present investigation revealed that supplementation of fenugreek (*Methi*) have significant effect on serum glucose and serum cholesterol levels in lactating Murrah buffaloes. Also, fenugreek (*Methi*) supplementation did not have any adverse effect on average daily milk yield and milk composition i.e. milk fat, 6% FCM, milk protein, SNF content, total solids and lactose in lactating Murrah buffaloes upto six months of lactation.

Keywords: Fenugreek, Murrah, Milk yield, Milk composition, Biochemical parameters

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The use of herbal galactogogues is known to have beneficial effect on milk production. Herbs are safe to use, cheap and easily available, has no side effect and no residual effect in milk in long term. Fenugreek (Trigonella foenum graecum), locally known as Methi, is one of the oldest herbs which grows throughout India and other parts of the world. Fenugreek (Trigonella foenum-graecum) is derived from a plant that belongs to the leguminous family. It is commonly known as Methi and is found in India, Middle East, North Africa and South Europe. The seeds are highly valued as food for man, cattle and sheep and to promote lactation and lactation performance in woman and ruminant. Fenugreek has been shown to have a positive effect on lactation performance in ruminants such as dairy cows, buffaloes and dairy goats (Nasser et al., 2013; EL-Basheir 2015; Degirmencioglu et al., 2016).

The World Health Organization encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature. Herbs are safe to use, cheap and easily available, has no side effect and no residual effect in milk in long term. The seeds of fenugreek are known to have hypoglycaemic, hypocholesterolemic, gastro and hepatoprotective and anti-oxidant properties. Fenugreek seeds also lowered serum triglycerides, total cholesterol and low-density lipoprotein cholesterol.

MATERIALS AND METHOD

In the present study, eighteen lactating Murrah buffaloes were selected and divided into three treatment groups having six in each on the basis of milk yield and body weight and parity following completely randomized design (CRD) from the herd of buffalo farm. Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. All the experimental lactating Murrah buffaloes used in the present study were kept under loose housing system. Prior approval was taken to conduct the present investigation from the Institutional Animal Ethics Committee. The daily allowance of concentrate mixture was offered to each animal at the time of milking in the morning and evening as per the Experimental diet *i.e.* Treatment 1 (control): Basal roughage-concentrate mixture diets as per ICAR (2013) feeding standards (T_1) , Treatment 2: Basal ration (control) supplemented with crushed fenugreek seeds (a)150 g per animal per day (T₂) and Treatment 3: supplemented group with crushed fenugreek seeds (a, 250 g per animal per day (T₃). All standard managemental practices and biosecurity measures were followed throughout the experiment. Prior to start of the experiment an adjustment period of 10 days was given to all the experimental buffaloes for acclimatization to the new house and feeding regimen. The experimental buffaloes were daily washed before milking. The animals

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were maintained under isomanagerial conditions and similar husbandry practices except the different feeding treatments. The study was conducted for a period from 10 days postpartum to 6 months of lactation. Before formulation of rations, the feed ingredients were analyzed for proximate composition (Table 1). Buffaloes were hand milked twice daily and milk yield was recorded by using digital weighing balance and the weekly average was calculated. Milk sample for composition analysis were first taken at tenth day after calving and thereafter at each fortnightly interval until the end of the experiment. These samples were taken during the morning and evening milking from each experimental buffalo and analysed with the help of Milko-Scan autoanalyser and the average of both times was calculated for estimation of milk quality in terms of Milk fat (%), Milk protein (%), Solid not fat content(%), Total solids (%) and 6% FCM = $0.308 \times$ Total milk $+ 11.54 \times$ Total fat.

From all the eighteen buffaloes belonging to the three experimental treatments, blood samples were collected aseptically during early morning hours before feeding and watering of buffaloes by jugular vein puncture. Approximately ten millilitre (ml) of blood was collected from each animal and transferred immediately into a set of sterile plastic tubes without anticoagulant. The contents of test tubes were held in slanting position for serum separation. The sera were centrifuged to remove the erythrocytes present, if any. The clear, non haemolysed sera were then collected in clean, dry and labelled vials. These sera were preserved under deep freezing in capped vials for further analysis. Blood sample were collected at the begining of the experiments i.e. day 0, and thereafter at monthly intervals that are on day 30, 60, 90, 120, 150 and 180th of experiment periods. The sera samples were analysed for estimation of biochemical parameters namely Blood glucose, Serum calcium, Serum cholesterol, Serum total protein, Serum globulin, Serum albumin, Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) using Automated Random Access Clinical Chemistry Analyser (EM Destiny 200) TM, Erba Diagnostics Mannheim GmbH). All the data were subjected to ANOVA using General Linear Model's procedure of SPSS-23 software (SPSS, 2019). The mean differences among different treatments were separated by Duncan's multiple range tests. Consequently, a level of (P<0.05) was used as the criterion for statistical significance (Duncan, 1955).

RESULTS AND DISCUSSION

Daily milk yield

The overall mean values of daily milk yield were

11.21, 11.11 and 11.46 in treatment groups T_1 , T_2 and T_3 , respectively. The result showed statistically no significant difference between treatments except at seventh week where mean values are higher in T_3 (Table 2 & Fig. 1). Kirar et al. (2018) reported that supplementation of fenugreek seeds @50 g and 100 g were accompanied by an increase in the daily milk yield and 90 days total milk yield compared to control group, but the increase was not significant in lactating Murrah buffalo. Trian (2003) suggested that increase in milk yield in fenugreek supplemented groups compared to control group might be due to the effect of diosgenin, which is a chemical compound in fenugreek seeds similar to the hormone oestrogen stimulating an increase in milk flow. Over a period of 180 days of experiment it was observed that the supplementation of fenugreek (methi) @ 150 g and 250 g did not have significant effect on average daily milk yield of the experimental buffaloes. Abo El-Nor et al. (2007) reported that supplementation of 200 g fenugreek seeds resulted in significant (P<0.05) increase in milk yield in lactating buffaloes. Maher and Nadya (2012) reported that feeding of 2 or 4% of Fenugreek seeds in Friesian cows and Nasser et al. (2013) reported that supplementation of 50 g and 100 g of fenugreek seed powder per cow per day in Sharabi cows significantly (P < 0.05) increased milk yield. Degirmencioglu et al. (2016) also reported that supplementation of 50 g ground fenugreek seeds significantly (P<0.01) increased mean daily milk production in Anatolian water buffaloes.

Milk composition

The overall average milk fat (%) in T_1, T_2 and T_3 groups were 6.93 ± 0.07 , 7.07 ± 0.05 and 7.09 ± 0.05 , respectively (Table 3). The overall average FCM (kg) in the three treatment groups were 12.14, 12.24 and 12.60 kg, respectively. The supplementation of fenugreek (Methi) @ 150 g and 250 g showed non-significant effect on milk composition in terms of milk fat, FCM, milk protein, SNF content, total solids and lactose and at each progressive sampling during the experimental period. Shah and Mir (2004) and El- Alamy et al. (2001) through their experiments found that feeding fenugreek seeds to buffaloes had no effects on milk composition. Kholif and Abdel-Gawad (2001) also reported that there was no significant difference on milk lactose content in fenugreek supplemented group compared to control group. Balgees et al. (2013) similarly reported that milk components (protein, lactose and SNF) showed inconsistent pattern by fenugreek seeds supplementation but there was significant (P<0.05) decrease in milk fat percentage. EL-Basheir (2015) also reported that supplementation of fenugreek Seeds @ 0, 2.5and 5% of the diet did not affect milk

Table 1. Proximate composition (%DM basis) of different feed ingredients fed to the experimental buffaloes

Ingredients	DM	CP	CF	EE	Ash	OM	NFE
Wheat straw	94.56	2.42	35.91	2.85	11.31	88.69	47.51
Berseem (Green)	22.95	15.73	27.51	3.11	8.55	91.45	53.1
Maize (Green)	24.91	10.43	26.2	4.29	11.14	88.86	47.94
Maize	89.02	9.17	2.31	3.65	1.08	98.92	83.79
Barley	93.49	9.76	7.89	1.81	4.83	95.17	75.71
Groundnut cake	95.56	45.71	8.65	6.29	5.15	94.85	34.2
Soyabean meal	89.45	45.91	4.69	2.93	7.37	92.63	39.1
Mustard cake	94.37	32.21	7.98	6.65	7.81	91.26	45.35
Fenugreek seed	89.81	28.40	9.30	7.14	3.28	96.72	51.88

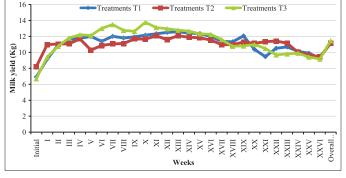


Fig. 1. Average milk yield (kg) of experimental buffaloes at weekly intervals under different dietary treatments

composition. Degirmencioglu et al. (2016) also reported that supplementation of 50 g of ground fenugreek seeds in Anatolian water buffaloes had no significant effect on SNF and protein percentages in milk. Contrary to the findings of the present study, Nasser et al. (2013) reported that supplemented fenugreek seed powder at a level 50 or 100 g/cow/day in Sharabi dairy cows resulted in significantly $(P \le 0.05)$ increased milk fat percentage.

Haemato-biochemical parameters

The serum glucose (mg/dl) and serum cholesterol were significantly higher in control group in comparison to treatment groups T₂ and T₃ with subsequent increase in levels of fenugreek seeds @ 150 g and 250 g, respectively (Table 4 and 5). The levels of serum glucose concentration and serum cholesterol followed inversely proportional trend with amount of fenugreek seed supplementation. The low blood glucose concentration due to fenugreek supplementation might be due to the presence of an amino acid called 4-hydroxy isoleucine in fenugreek seeds which appears to act on pancreatic β -cells to increase production of insulin in the body. Higher amounts of insulin production may decrease the sugar that stays in the blood (Schryver, 2002). The findings of Raju et al. (2001) and Devi et al. (2003) that the ability of fenugreek seeds to modulate key glucose metabolizing enzymes such as hexokinase (glycolysis), glucose-6-phosphatase or fructose-1, 6bisphosphatase (gluconeogenesis) might be considered as a possible mechanism. The average total protein, serum

Period (Week)		Treatments	
	T ₁	T ₂	T ₃
Initial	6.91±0.66	8.21±0.74	6.70±1.00
Ι	9.15±0.52	10.98±0.66	9.41±0.83
II	10.91±0.65	11.03±0.76	10.75±0.60
III	11.60±0.67	11.07±0.60	11.81±0.51
IV	11.78 ± 0.91	11.68 ± 0.51	12.20 ± 0.47
V	11.98 ± 0.78	10.28±1.24	12.11±0.39
VI	11.38 ± 1.10	10.85 ± 0.87	13.00±0.41
VII	$12.01{\pm}0.70^{\text{ab}}$	$11.06{\pm}0.51^{a}$	13.48±0.52 ^b
VIII	11.80±0.99	11.10±0.31	12.75±0.78
IX	11.93±0.85	11.68±0.43	12.63±0.83
Х	12.15±0.63	11.65±0.47	13.76±1.20
XI	12.33±0.68	12.06 ± 0.46	13.11±0.38
XII	12.50 ± 0.62	11.58 ± 0.36	12.95±0.60
XIII	12.58 ± 0.81	12.08 ± 0.31	12.78 ± 0.48
XIV	12.41 ± 0.89	11.88 ± 0.82	12.66±0.51
XV	12.36±0.88	11.83±0.57	12.31 ± 0.60
XVI	11.98 ± 0.57	11.53 ± 0.47	12.26±0.41
XVII	11.38 ± 1.05	10.96 ± 0.54	11.60 ± 0.68
XVIII	11.31±0.91	10.95 ± 0.41	10.76±0.36
XIX	12.06 ± 0.99	11.28 ± 0.54	10.80 ± 0.50
XX	10.38 ± 1.62	11.11 ± 0.43	10.98 ± 0.30
XXI	9.46±1.51	11.33 ± 0.52	10.50 ± 0.45
XXII	10.51 ± 1.44	11.41 ± 0.72	9.68 ± 0.40
XXIII	10.68 ± 1.30	11.15±0.63	9.80±0.24
XXIV	10.13 ± 1.09	10.00 ± 0.46	9.90 ± 0.32
XXV	9.88 ± 0.94	9.66±0.24	9.41±0.20
XXVI	9.28 ± 0.80	9.43±0.19	9.16±0.24
Overall mean	11.21±0.76	11.11±0.39	11.46±0.27
The mean value	ues with differe	ent superscripts	in a row differ

Table 2. Average milk yield (kg) of experimental buffaloes at weekly intervals under different dietary treatments

The mean values with different superscripts in a row differ significantly (P < 0.05)

globulin, serum albumin, SGOT and SGPT values were non-significant among all the treatment groups.

CONCLUSION

The supplementation of Fenugreek (Methi) in lactating Murrah buffaloes @ 150 g and 250 g has no adverse effect on milk production as well as on milk composition. The supplementation of Fenugreek significantly affects the serum cholesterol levels in the lactating Murrah buffaloes.

Period (Fortnigh	tly)	Treatments	
	T ₁	T ₂	T ₃
Initial	8.10±0.02	8.08±0.01	8.14±0.07
Ι	7.05 ± 0.20	7.17 ± 0.04	7.08 ± 0.23
Ι	6.96±0.14	7.15±0.04	7.08 ± 0.26
III	6.55±0.12	6.90 ± 0.05	6.85±0.15
IV	6.41±0.10	6.51±0.10	6.56 ± 0.05
V	6.41 ± 0.01	6.68 ± 0.08	6.68 ± 0.74
VI	6.43±0.01	6.50±0.10	6.68 ± 0.08
VII	6.55±0.12	6.65 ± 0.10	6.66±0.12
VIII	6.96±0.14	7.15 ± 0.04	7.08 ± 0.26
IX	6.96 ± 0.04	7.08 ± 0.26	7.15±0.04
Х	7.05 ± 0.20	7.17±0.04	7.08 ± 0.26
XI	6.93±0.12	7.06 ± 0.02	7.15 ± 0.04
XII	7.15±0.04	7.36 ± 0.08	7.27±0.12
XIII	7.55±0.16	7.60 ± 0.17	7.70±0.12
Overall mean	6.93 ± 0.07	7.07 ± 0.05	7.09 ± 0.05

 Table 3.
 Average fat (%) in milk of experimental buffaloes at fortnightly intervals under different dietary treatments

The mean values with different superscripts in a row differ significantly (P < 0.05)

 Table 4.
 Average serum glucose (mg/dl) values of experimental buffaloes at monthly intervals under different dietary treatments

Period (Month)	Treatments			
	T ₁	T_2	T ₃	
Initial	64.38±0.29	64.40±0.30	64.28±0.29	
Ι	67.55°±0.17	66.37 ^b ±0.17	$64.17^{a}\pm0.25$	
II	$68.01^{\circ}\pm0.07$	64.84 ^b ±0.17	$63.12^{a}\pm0.07$	
III	69.59 ^b ±0.15	69.43 ^b ±0.14	$66.42^{a}\pm0.14$	
IV	68.42°±0.11	67.01 ^b ±0.04	$64.46^{a}\pm0.13$	
V	69.38°±0.14	67.24 ^b ±0.08	$65.02^{a}\pm0.03$	
VI	69.31°±0.08	68.38 ^b ±0.11	65.25 ^ª ±0.11	
Overall mean	$67.92^{\circ}\pm0.03$	$66.97^{b} \pm 0.03$	$64.67^{a} \pm 0.05$	

The mean values with different superscripts in a row differ significantly (P<0.05)

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 Table 5.
 Average serum cholesterol ((mg/dl)) values of experimental buffaloes at monthly intervals under different dietary treatments

Period (Month)	Treatments			
	T ₁	T ₂	T ₃	
Initial	88.46±0.38	87.85±0.45	88.83±0.53	
Ι	99.8±0.70	99.23±0.28	98.62±0.18	
II	$106.97^{b} \pm 0.78$	101.95 ^ª ±0.73	$100.82^{a}\pm 0.43$	
III	104.16 ^b ±0.93	102.73 ^b ±0.55	100.21ª±0.69	
IV	104.98°±0.53	98.48 ^b ±0.23	$94.89^{a}\pm0.69$	
V	$101.67^{b} \pm 0.55$	$88.86^{a} \pm 0.99$	$87.42^{a}\pm 0.53$	
VI	98.48 ^b ±0.23	87.01 ^ª ±0.46	87.64 ^ª ±0.53	
Overall mean	$100.65^{\circ}\pm 0.20$	$95.16^{b} \pm 0.27$	$94.06^{a} \pm 0.10$	

The mean values with different superscripts in a row differ significantly (P < 0.05)

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