



# The effect of fenugreek seed (*Trigonella foenum-graecum*) supplementation on the performance and milk yield characteristics of dairy goats

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**Abstract.** The use of fenugreek seed as a galactagogue has been investigated in ruminants. However, only a few studies have analyzed the effects of fenugreek seed on the milk fatty acid composition. To the authors' best knowledge, none of these studies have investigated the effects of fenugreek seed on milk volatile compounds. The objective of this study, which lasted 8 weeks, was to determine the effects of fenugreek seed supplementation on the performance, milk fatty acid profile, and milk volatile profile of goats. A total of 20 Turkish Saanen dairy goats that were mid-lactation were used in the study. This study was carried out with two homogeneous groups: the control group (CON) and fenugreek group (FG). Both groups were fed a total mixed ration (TMR), but the FG treatment had their diet supplemented with 0.1 kg d<sup>-1</sup> of fenugreek seed per animal. The feed intake, live weight, milk yield, milk chemical composition, serum cholesterol and triglyceride concentration, milk fatty acid profile, and milk volatile compound profile were determined. The feed intake, live weight gain, and milk yield of the goats did not significantly differ between the CON and FG treatments ( $P > 0.05$ ), whereas the milk fat concentration increased ( $P = 0.05$ ) and the serum cholesterol and triglyceride concentrations decreased ( $P < 0.05$ ) in the FG treatment. The unsaturated fatty acid concentration in milk ( $P < 0.05$ ) was also enhanced in the FG treatment. In addition, the FG treatment increased 2-propanone, 2-butanone, benzene, 1-methyl-3-(1-methylethyl), and eucalyptol concentrations in milk ( $P < 0.05$ ). In conclusion, the supplementation of goats' diets with fenugreek seed during the mid-lactation period did not affect their performance and improved the unsaturated fatty acid profile of their milk. Of particular importance, we have also detected an increase in the 2-propanone concentration in milk following fenugreek supplementation.

## 1 Introduction

The yield and composition of milk are important with respect to the nutritional quality of milk and milk-derived products. Goat milk is more likely to be used to make cheese. The milk fat concentration, milk fatty acid profiles, and milk flavor are changed by dietary factors. More specifically, the forage-to-concentrate ratio, the particle size, lipid supplementation, and the use of probiotics and plant extracts are among the dietary factors affecting the quality of dairy products (Pulina et al., 2008). Plants produce many different bioactive sub-

stances as a part of an evolutionarily developed defense mechanism against insects and herbivores (War et al., 2012). However, plants or plant parts, such as leaves, flowers, roots, and seeds, also contain bioactive compounds that are used in the nutrition of ruminant animals to improve their performance and milk quality (Kohalif et al., 2016). Plants' bioactive substances, like saponins, tannins, and flavonoids, have the potential to be used as synthetic or chemical feed additives in order to modify rumen fermentation characteristics (Patra and Sxena, 2009). Thus, these bioactive compounds that occur naturally in plants can potentially be used in or-

ganic animal production to increase milk yield, and the interest in the use of such plants to increase milk production is increasing. In addition, many plant species have been reported to contain bioactive ingredients with galactogenic effects (Bharti et al., 2012; Tabares et al., 2014). Among these plants, fenugreek is the most widely used (Zuppa et al., 2010; Bahmani et al., 2016). It is known that Arabic and Indian women use fenugreek seed to increase their milk production after delivery (Al-Shaikh et al., 1999; Tiran, 2003; Ravi and Joseph, 2020). There are also a few publications on using fenugreek seed to increase the milk yield of farm animals (Hassan et al., 2012; Balgees et al., 2013; Degirmencioglu et al., 2016).

Fenugreek belongs to the genus *Trigonella*, which is in the legume family. This genus is cultivated in the Mediterranean Basin and includes 50 different species (Kumar et al., 2020). Fenugreek is mainly cultivated to be used as a spice and forage for animals in different countries, and its seeds have a strong flavor and aroma (Acharya et al., 2006; Kaviarasan et al., 2006). Fenugreek seed contains proteins, amino acids, flavonoids, tannins, alkaloids, mucilage, and saponins (Yadav et al., 2011). Saponins are secondary compounds and can alter the rumen microbial community and fermentation, especially by limiting the protozoa population (Patra and Saxena, 2009). In addition, it has been reported that plants with high concentrations of saponin alter the rumen volatile fatty acid concentration (Guyader et al., 2017). Moreover, the short-term supplementation of tea saponin has been said to have the potential to increase the level of unsaturated fatty acids in cow milk (Wang et al., 2019).

Diosgenin, which is found in fenugreek seed, is a form of steroidal saponin, and it is thought to stimulate the release of hormones that increase milk production. It has been suggested that diosgenin stimulates growth hormone (GH) release (Graham et al., 2008; Smith, 2014) and prolactin secretion, which has a galactopoietic effect, by showing an estrogen-like effect (Hassan et al., 2012). In addition to the abovementioned effects, fenugreek seed supplementation might change the volatile compound profile of milk due to its strong aroma. However, in the literature, none of the existing studies have evaluated the effects of fenugreek supplementation on the volatile compound profile in milk; moreover, to the best of our knowledge, only one study has evaluated the effects of fenugreek seed on the milk fatty acid profile in dairy cows (Shah and Mir, 2004). The hypothesis of the current study was that the addition of fenugreek seed to dairy goat rations would enhance milk yield and milk quality. Thus, this work aimed to investigate the effects of fenugreek seed supplementation on the performance, milk yield, milk fatty acid composition, and milk volatile compound profile of dairy goats.

## 2 Materials and methods

The protocol for animal use and implementation was confirmed by the ethical guidelines in force at the University of Çanakkale Onsekiz Mart (protocol no. 2017/02-10). The study was conducted at the Research and Application Farm of the Faculty of Agriculture at the University of Çanakkale Onsekiz Mart.

### 2.1 Study animals and diet

For this work, 20 Turkish Saanen goats ( $39.54 \pm 0.3$  kg live weight) were selected from the abovementioned Research and Application Farm. The study was conducted for 8 weeks. The parities of the experimental goats varied from 1 to 3, and they were in the middle stage of lactation ( $110 \pm 7$  DIM, days in milk;  $1.7 \pm 0.03$  L d<sup>-1</sup>). The goats were randomly allocated into two groups ( $n = 10$  goats per group) and housed in individual pens (1.5 m × 1.5 m). The control (CON) and fenugreek (FG) treatments were both fed with a basal ration, but animals in the FG treatment also had their diet supplemented with fenugreek seed in addition to the basal ration. The basal diet consisted of a total mixed ration (TMR) prepared for lactating goats, and it was obtained from a feed factory (Rasyonel Food Agriculture and Livestock Marketing Ind., Afyonkarahisar, Turkey) in vacuum packs (40 kg per pack). The forage-to-concentrate ratio of the TMR was 60 : 40. The TMR contained maize silage, meadow hay, molasses, maize grain, sunflower meal, and goat milk concentrate feed (wheat bran, maize, rice bran, oat, sodium bicarbonate, vitamin, premix, salt, rice bran, dried distillers' grain and solubles – DDGS, canola meal, and razmol). The composition of the TMR was guaranteed by the feed factory.

### 2.2 Methods

The daily amount of basal diet (TMR) offered to the goats (CON and FG) used in this work was determined according to NRC (2007) recommendations for the mid-lactation period (1.96 kg DM and 3.74 Mcal ME). The goats in the FG treatment were fed with the TMR and 0.1 kg d<sup>-1</sup> of fenugreek seed per goat. Individually, animals in the FG treatment were fed with fenugreek seed (50 g per meal) just before the morning (08:30 LT, local time, UTC+3) and evening (16:30 LT) TMR feeds, in order to ensure the consumption of the same amount of seed at each meal. The goats' feed intake was recorded daily. The feed samples were collected at 15 d intervals ( $N = 4$ ), and they were ground (1 mm mesh) and analyzed for dry matter (DM), ether extract (EE), crude protein (CP), and ash according to the AOAC (1990) procedures (ID nos. 924.05, 920.29, 955.04, and 924.05 for DM, EE, CP, and ash, respectively). Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were analyzed according to the procedure suggested by Van

**Table 1.** The chemical composition of the total mixed ration (TMR) and fenugreek seed.

Item	TMR N = 4		Fenugreek seed N = 4	
	$\bar{x}$	SD	$\bar{x}$	SD
DM	51.00	0.63	91.00	0.55
CP	16.81	0.37	29.18	0.39
EE	3.50	0.29	3.91	0.17
NDF	47.98	3.82	13.75	2.15
ADF	39.84	2.57	10.55	1.93
ADL	7.28	0.36	2.26	0.33
Ash	8.99	0.18	3.94	0.10
ME	2.62	0.03	2.70	0.01

The abbreviations used in the table are as follows: DM – dry matter (%), CP – crude protein (% DM), EE – ether extract (% DM), NDF – neutral detergent fiber (% DM), ADF – acid detergent fiber (% DM), ADL – acid detergent lignin (% DM), ME – metabolizable energy (Mcal ME kg<sup>-1</sup> DM), and SD – standard deviation.

Soest et al. (1991). The chemical compositions of the TMR and fenugreek seed are shown in Table 1.

The goats were weighed at a weekly interval with an electronic bascule that was sensitive up to 20 g. The blood samples (10 mL) were collected 2 h after morning feeding in the middle (fourth week) and at the end (eighth week) of the study via jugular venipuncture into plain Vacutainer tubes without anticoagulants (Becton, Dickinson and Company, USA). The serum separation process from the blood samples was performed by centrifugation at 3000 g for 15 min (WHO, 2002), and the serum samples were stored at –20 °C until the day of cholesterol and triglyceride analysis. The concentrations of serum cholesterol (CHOD-PAP) and triglyceride (GPO-PAP) were determined with a spectrophotometer (UV-mini 1200, Shimadzu, Kyoto, Japan) using a commercial kit (DiaClinica Diagnostic Industry, Turkey).

The milk yield of the individual goats was determined at a weekly interval (09:00–18:00 LT) using a milking machine, and the milk samples were taken for chemical composition (50 mL per goat; a mixed equal volume of evening and morning milk). Milk was analyzed for “solids-non-fat” (SNF), protein, fat, and lactose using an ultrasonic milk analyzer (Milk-Lab Minor<sup>®</sup>, UK Ltd.). The dry matter content of the milk samples was calculated using the multiple solids-non-fat and fat content. The milk samples for fatty acid (FA) and volatile compound profile analyses were collected at the end of the study from 20 goats from both the evening and morning milking. A 50 mL milk sample was then kept at –20 °C until free fatty acid analysis was carried out.

Numerous analyses were undertaken in this work. Milk yields corrected for 3.5 % fat (referred to as fat-corrected milk – FCM) were calculated (in kg d<sup>-1</sup>) according to the following formula developed by Sklan et al. (1994): FCM (kg) = [0.432 + (0.1625 × milk fat

content) × milk yield]. Feed efficiency (FE) was calculated as follows: FE = milk yield (kg)/dry matter intake (kg). Energy-corrected milk (ECM) was calculated according to NRC (2001): ECM (kg d<sup>-1</sup>) = 0.03246 × milk yield + (12.86 × fat yield) + (7.04 × protein yield). The milk energy value was calculated according to Baldi et al. (1992): ME (kcal kg<sup>-1</sup>) = 203.8 + (8.36 × fat %) + (6.29 protein %).

### 2.3 Free fatty acid analysis

Milk fatty acid extraction was carried out according to the method used by Luna et al. (2005). The methylation processes of fatty acids were performed according to TS EN ISO 12966-1 (Anonymous, 2015). Fatty acid composition was determined using a gas chromatograph (model 7820, Agilent Technologies Inc., Wilmington, DE, USA) equipped with a DB-23 capillary column (60 m × 0.25 mm × 0.25 μm film thickness) and a flame ionization detector (FID). The percentage of individual fatty acids was expressed as a percentage of total fatty acids. The fatty acid profiles of the TMR and fenugreek seed are presented in Table 2.

### 2.4 Volatile compounds

The volatile compounds in the milk samples were isolated via solid-phase microextraction (SPME) and identified using gas chromatography–mass spectrometry (GC-MS) (Güneşer and Yüceer, 2011). A total of 5 mL of milk and 1 g of NaCl were added to a 40 mL vial with a polytetrafluoroethylene (PTFE)/silicone septa hole cap (Supelco, Bellafonte, US) and kept in a 40 °C water bath for 20 min in order to stabilize the volatiles in the headspace. The fiber (2 cm, 50/30 μm, divinylbenzene/Carboxen/polydimethylsiloxane – DVB/CAR/PDMS) was immersed in the headspace of the vial, kept in a 40 °C water bath for 20 min, and then injected into the GC-MS (model 6890, Agilent Technologies Inc., Wilmington, DE, USA). A DB-5MS column (60 m × 0.250 mm i.d. × 0.25 μm film thickness; J&W Scientific, Folsom, CA, US) was used to separate the volatiles.

The GC-MS conditions were as follows: 1.5 mL min<sup>-1</sup> carrier gas (He) flow; splitless injection mode; 40 °C initial oven temperature, held for 1 min, ramped to 250 °C at 7 °C min<sup>-1</sup>, and held for 15 min; 280 °C capillary interface temperature; 70 eV ionization energy; 35–350 amu mass range; and a 4.45 scans<sup>-1</sup> scan rate. The volatiles were identified using spectral libraries (NIST, 2008 and WILEY, 2005).

### 2.5 Statistical analysis

The data regarding nutrient intake (DM, CP, and ME intake), live weight, milk yield, milk composition, and serum parameters were analyzed using repeated measurement variance analyses in the statistical package of SAS Institute Inc. (1999). The statistical model included groups (CON and

**Table 2.** Fatty acid (FA) profile of TMR and fenugreek seed consumed by goats.

Fatty acid (grams per 100 g of total FA)	TMR		Fenugreek	
	$\bar{x}$	SD	$\bar{x}$	SD
C13:0	nd	nd	0.18	0.021
C14:0	0.32	0.051	2.60	0.120
C14:1	0.04	0.004	0.10	0.011
C15:1	nd	nd	0.01	0.003
C16:0	17.36	0.920	10.23	0.690
C16:1	0.49	0.043	0.09	0.013
C17:0	0.039	0.012	0.28	0.042
C17:1 <i>cis</i> -10	0.03	0.001	0.12	0.020
C18:0	2.30	0.152	4.46	0.510
C18:1n9c	24.96	0.981	17.69	1.100
C18:2n6c	48.70	2.010	40.88	1.942
C20:0	0.75	0.163	0.11	0.024
C18:3n3	2.70	0.167	19.28	0.873
C20:1 <i>cis</i> -11	0.63	0.032	0.31	0.062
C20:2 <i>cis</i> -11,14	0.01	0.007	nd	nd
C22:0	0.02	0.006	nd	nd
C20:3n3 <i>cis</i> -11,14,17	0.05	0.009	0.21	0.020
C20:4n6	0.05	0.008	nd	nd
C20:5n3 <i>cis</i> -5,8,11,14,17	0.06	0.002	nd	nd
C22:6n3	1.41	0.062	0.87	0.114
C22-1n9	nd	nd	0.10	0.005
C24:1	nd	nd	2.47	0.132

The abbreviations used in the table are as follows: nd – not detected, and SD – standard deviation.

FG), observation day, parity, birth type, number of days in lactation, and their interactions as fixed factors, and the individual goats nested within the group were included as a random effect. The milk fatty acid profile and milk volatile compound profile data were analyzed with an analysis of variance using group, and milk yield was a covariate. A Tukey test was used in the post hoc analysis, with a significance level of  $P \leq 0.05$ .

### 3 Results

The yield and chemical composition of the goats' milk are presented in Table 3. DM, CP, and ME intake was not affected by the fenugreek seed supplementation. The live weight and milk yield of the goats did not change between the groups. However, the experimental groups differed with respect to 3.5 % FCM and ECM yield ( $P < 0.001$ ). The 3.5 % FCM and ECM yield values of the FG treatment were higher than those of the CON treatment (Table 3). The milk fat concentration and fat yield were higher in the FG treatment than in the CON treatment ( $P < 0.05$ ). The protein, lactose, and SNF contents of milk were found to be similar in both groups. Milk lactose and protein yield varied between the groups ( $P < 0.05$ ). Higher total solid and energy values were found in the FG treatment than in the CON treatment ( $P < 0.05$ ). The difference in feed efficiency

between the FG and CON treatments was not significant. The 3.5 % FCM / DMI (fat-corrected milk/dry matter intake) and ECM / DMI (energy-corrected milk/dry matter intake) values were affected by fenugreek seed supplementation ( $P < 0.001$ ). Higher serum cholesterol and triglyceride concentrations were found in the FG treatment than in the CON treatment ( $P < 0.01$ ).

The fatty acid composition of the goats' milk is presented in Table 4. Palmitic acid (C16:0) had the highest concentration of fatty acid (32.97 %), followed by oleic (C18:1n9c) acid (28.01 %), elaidic (C18:1n9t) acid (14.00 %), and myristic (C14:0) acid (8.76 %). Fenugreek seed supplementation decreased the concentration of myristic acid ( $P < 0.0505$ ), whereas the concentrations of arachidic (C20:0) acid and margaric acid (C17:0) were increased ( $P < 0.05$ ). Oleic acid and elaidic acid were higher in the FG treatment than in the CON treatment ( $P < 0.05$ ). Fenugreek seed supplementation significantly increased the arachidonic acid (C20:4n6) concentration ( $P < 0.01$ ). There were no differences between saturated fatty acid (SFA), medium-chain fatty acid (MCFA), polyunsaturated fatty acid (PUFA), and monounsaturated fatty acid (MUFA) concentrations in the groups.

The volatile compound profile of fenugreek seed is presented in Table 5. A total of 24 volatile compounds were identified in the fenugreek seed used in this study.

**Table 3.** The least squares mean ( $\bar{x}$ , LSM) and the standard error of the mean (SEM) for the effects of fenugreek supplementation on performance, milk composition, and serum parameters in goats.

Item	CON N = 20		FG N = 20		P value
	$\bar{x}$	SEM	$\bar{x}$	SEM	
DMI (kg d <sup>-1</sup> )	1.69	0.079	1.58	0.075	0.293
CPI (g d <sup>-1</sup> )	286.08	12.742	266.56	13.366	0.293
MEI (Mcal ME d <sup>-1</sup> )	4.40	0.196	4.11	0.205	0.293
LW (kg)	40.7	0.500	41.1	0.561	0.581
Milk yield (kg d <sup>-1</sup> )	1.41	0.049	1.36	0.055	0.507
<b>Milk</b>					
3.5 % FCM (kg d <sup>-1</sup> )	1.28	0.032	1.41	0.036	0.008
ECM (kg d <sup>-1</sup> )	1.09	0.019	1.19	0.0203	0.001
Fat (%)	3.86	0.152	4.41	0.166	0.012
Fat (kg d <sup>-1</sup> )	0.51	0.013	0.60	0.013	0.000
Protein (%)	2.86	0.033	2.94	0.036	0.076
Protein (kg d <sup>-1</sup> )	0.39	0.028	0.43	0.029	0.030
Lactose (%)	4.31	0.049	4.43	0.053	0.069
Lactose (kg d <sup>-1</sup> )	0.59	0.042	0.61	0.044	0.014
Total solids (%)	11.80	0.238	12.58	0.262	0.022
Non-fat solids (%)	7.81	0.090	8.03	0.098	0.069
Milk energy value (kcal kg <sup>-1</sup> )	253.74	0.598	259.33	0.669	0.000
Feed efficiency	0.82	0.032	0.88	0.035	0.148
3.5 % FCM / DMI	0.77	0.021	0.91	0.024	0.000
ECM / DMI	0.70	0.017	0.81	0.019	0.000
<b>Serum concentration</b>					
Cholesterol (mg dL <sup>-1</sup> )	70.04	1.928	62.21	1.971	0.006
Triglyceride (mg dL <sup>-1</sup> )	55.21	0.786	51.98	0.803	0.005

The abbreviations used in the table are as follows: DMI – dry matter intake, CPI – crude protein intake, MEI – metabolizable energy intake, LW – live weight, FCM – fat-corrected milk, and ECM – energy-corrected milk.

Table 6 shows the volatile compounds detected in the goat milk samples. A total of 19 volatiles were found in all milk samples. Volatiles such as 2-propanone, 2-butanone ( $P < 0.05$ ), and oxime-methoxy-phenyl were found in high proportions in the identified compounds ( $P > 0.05$ ). The ratios of 2-propanone, 2-butanone, pinene, cymene, and eucalyptol in the FG treatment were found to be higher ( $P < 0.05$ ) than those in the CON samples (Table 6).

#### 4 Discussion

Fenugreek supplementation did not affect dry matter intake (DMI) or live weight (LW) in the goats used in this experiment. However, several studies have reported that the supplementation of fenugreek seed increases the DMI of farm animals (Hassan et al., 2012; Balgees et al., 2013; Değirmencioğlu et al., 2016). The increase in feed intake observed in previous studies has been explained by the steroidal saponin fractions isolated from fenugreek seed (Petit et al., 1995; Roberts, 2011). In addition, Abo El-Nor et al. (2007) re-

ported that the steroidal saponin fractions of fenugreek seed increase the motivation for feed intake by stimulating the hunger center of the brain. However, some studies have reported that the supplementation of fenugreek seed has no effect on feed intake or live weight gain (Şahin et al., 2003; Anmar and Al-Wazeer, 2017), which is supported by our findings. Interestingly, in studies examining the effects of fenugreek seed on the growth performance of lambs, no effect on feed intake or live weight gain was detected. The differences in the results obtained from these studies may be due to the differences in the level of fenugreek seed used or the animals used (different physiological periods). Higher milk yields with the supplementation of fenugreek seed in dairy goats and ewes at different levels have been reported in previous studies (Hassan et al., 2012; Balgees et al., 2013; El-Tarabany et al., 2018; Hasin et al., 2019), but no significant effect on milk yield was found in the current work (Table 3). The increase observed in milk yield with the supplementation of fenugreek seed is associated with the fact that diosgenin in fenugreek seed increases milk flow via an estrogen-like

**Table 4.** The least squares mean ( $\bar{x}$ , LSM) and the standard error of the mean (SEM) for the fatty acid (FA) profile of goat milk.

Fatty acid (G/100 g of total FA)	CON	FG	SEM	P value
	N = 20	N = 20		
C4:0	1.83	1.76	0.053	0.359
C6:0	1.83	1.75	0.091	0.532
C8:0	6.89	6.02	0.381	0.119
C10:0	0.15	0.37	0.080	0.067
C12:0	3.02	2.41	0.221	0.064
C13:0	0.06	0.06	0.004	0.656
C14:0	10.44	8.76	1.700	0.050
C14:1	0.22	0.24	0.030	0.614
C15:0	0.78	0.84	0.030	0.197
C15:1 <i>cis</i> -10	0.20	0.21	0.022	0.660
C16:0	36.15	32.97	2.74	0.440
C16:1	0.97	1.11	0.061	0.117
C17:0	0.41	0.47	0.018	0.022
C17:1 <i>cis</i> -10	0.19	0.20	0.008	0.228
C18:0	8.06	9.53	0.050	0.127
C18:1 <i>n9t-trans</i>	9.12	14.00	0.266	0.010
C18:1 <i>n9c</i>	25.54	28.01	1.312	0.039
C18:2 <i>n6c</i>	1.96	1.87	0.064	0.360
C18:3 <i>n3</i>	0.62	0.51	0.063	0.218
C20:0	0.09	0.11	0.006	0.017
C21:0	0.19	0.24	0.027	0.286
C20:3 <i>n3cis</i> -11-14,17	0.13	0.16	0.001	0.000
C20:5 <i>n3cis</i> -5,8,11,14,17	0.03	0.05	0.009	0.201
C20:4 <i>n6</i>	0.02	0.03	0.001	0.002
C22:0	0.11	0.12	0.008	0.507
C22:2 <i>cis</i> -13,16	0.03	0.04	0.004	0.446
C24:0	0.07	0.05	0.008	0.283
MCFA	10.06	8.80	0.603	0.154
SFA	44.61	44.11	5.802	0.952
MUFA	22.80	26.92	3.770	0.426
PUFA	1.63	1.37	0.310	0.590

The abbreviations used in the table are as follows: MCFA – medium-chain fatty acids (C8:0 + C10:0 + C12:0), SFA – saturated fatty acids (C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C24:0), MUFA – monounsaturated fatty acids (C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1 + C24:1), and PUFA – polyunsaturated fatty acids (C18:2 + C18:3 + C20:2 + C20:5 + C22:2).

effect (Tiran, 2003). Our study differs from the abovementioned literature in that slightly lower fenugreek seed supplementation ( $100 \text{ g d}^{-1}$ ) was used; moreover, the current study was conducted using goats that were mid-lactation, whereas the aforementioned studies were conducted on animals during the early lactation period. Contrary to this, an increase in milk yield was shown by Hassan et al. (2012), who supplemented the diet of Sudanese desert ewes with fenugreek seed ( $2.5\text{--}5 \text{ g kg}^{-1}$  live weight) for 7 weeks during the mid-lactation period. The authors claimed that fenugreek seed supplementation increased the serum prolactin and thyroid-stimulating hormone (TSH) concentrations, which had a detrimental effect on milk production. Conversely, according to results obtained from studies examining the effects of fenugreek seed on breast milk production in humans, it has been reported that the use of fenugreek seed in early lactation is more effective than during later lactation stages, as fenugreek seed did not affect breast milk production and serum prolactin levels in the later lactation stage (El Sakka et al.,

**Table 5.** Volatile compound profile of the fenugreek seed consumed by goats.

Volatile compound	Concentration (%)		
	RT	$\bar{x}$	SD
Propanone (acetone)	3.91	1.56	0.064
2-Butanone	4.63	0.45	0.049
( <i>E</i> )-2-Methyl-2-butenal	6.80	7.67	0.092
1-Pentanol	7.20	0.79	0.049
2-Buten-1-ol, 2-methyl	7.40	0.88	0.071
Hexanal	8.01	2.37	0.035
1-Hexanol	9.58	2.31	0.071
<i>p</i> -Xylene	9.75	0.05	0.007
Oxime-, methoxy-phenyl-	9.98	4.42	0.042
2-Heptanone	10.11	0.21	0.014
Trisiloxane, 1,1,3,3,5,5-hexamethyl	11.24	1.52	0.014
Pinene	11.34	1.83	0.085
Hexanoic acid	12.02	0.32	0.092
Myrcene	12.54	0.46	0.042
1-Hexanol, 2-ethyl-	13.44	0.09	0.006
Benzene, 1-methyl-3-(1-methylethyl)	13.51	0.39	0.021
D-Limonene	13.64	0.67	0.071
Eucalyptol	13.75	1.04	0.141
2-Nonanone	14.92	0.05	0.014
$\alpha$ -Terpinolene	15.15	0.18	0.071
Nonanal	15.26	0.34	0.078
Phenylethyl alcohol	15.56	0.42	0.028
Camphor	16.50	0.12	0.021
Terpinene	20.49	0.54	0.078

The abbreviations used in the table are as follows: RT – retention time and SD – standard deviation.

2014; Abdou and Fahey, 2018). These contradictory results may be due to species or breed characteristics as well as differences in the lactation peaks between species or breeds.

Fenugreek seed supplementation leads to an increase in the 3.5 % FCM and ECM values observed in the FG treatment, possibly due to the significantly higher milk fat concentration measured in this treatment (Table 3). The higher milk protein and milk fat concentrations also lead to higher milk fat and milk protein yield in the FG treatment than in the CON treatment. The findings of other studies regarding the effects of fenugreek seed supplementation on milk fat are varied. Balgees et al. (2013) and El-Tarabany et al. (2018) state that a significantly lower milk fat concentration was found with fenugreek seed supplementation, whereas Abo El-Nor et al. (2007) and Değirmencioglu et al. (2016) report that fenugreek seed supplementation did not affect the milk fat concentration. Previous reports of enhanced feed efficiency and 3.5 % FCM / DMI with supplementation of fenugreek seed (Allam et al., 1999; Abo-El-Nor et al., 2007; Abou-Elenin et al., 2016) are also in agreement with the findings of the current work. Fenugreek seed supplementation

**Table 6.** The least squares mean ( $\bar{x}$ , LSM) and the standard error of the mean (SEM) for the volatile compound profile (%) of goat milk.

Volatile compound	CON N = 20		FG N = 20		P value
	$\bar{x}$	SEM	$\bar{x}$	SEM	
2-Propanone (acetone)	3.95	0.742	6.79	0.920	0.023
2-Butanone	0.62	0.076	5.70	0.872	0.000
Butanoic acid EE	1.66	0.427	1.05	0.544	0.391
<i>p</i> -Xylene	0.62	0.353	0.25	0.043	0.514
Oxime-, methoxy-phenyl-	6.05	0.517	5.50	0.641	0.505
2-Heptanone	0.30	0.041	0.25	0.045	0.469
Hexanoic acid, methyl ester	0.72	0.192	0.22	0.025	0.125
Trisiloxane, 1,1,3,3,5,5-hexamethyl	1.35	0.134	1.65	0.108	0.094
Pinene	0.55	0.057	0.20	0.048	0.000
Hexanoic acid	–	–	–	–	–
1-Hexanol, 2-ethyl-	0.24	0.029	0.27	0.036	0.443
Cymene (benzene, 1-methyl-3-(1-methylethyl))	0.21	0.069	0.60	0.083	0.001
D-Limonene	0.39	0.079	0.45	0.096	0.653
Eucalyptol	2.33	0.511	5.26	0.618	0.001
$\alpha$ -Terpinolene	0.71	0.113	0.56	0.146	0.417
Nonanal	0.19	0.022	0.16	0.026	0.348
Octanoic acid	1.37	0.519	0.57	0.073	0.389
Camphor	0.26	0.049	0.32	0.053	0.420
Octanoic acid, ethyl ester	0.53	0.200	0.15	0.042	0.422
Terpinene	0.48	0.142	0.34	0.142	0.489

“–” denotes not detected.

increased milk energy, 3.5 % FCM / DMI, and ECM / DMI values.

Goats fed a diet supplemented with fenugreek seed showed a significant decrease in serum cholesterol and triglyceride concentrations (Table 3). Our study is in agreement with the findings of Petit et al. (1993), Al-Hobori and Raman (1998), and El Tarabany et al. (2018), who reported that fenugreek seed supplementation has been noted to reduce the blood cholesterol and triglyceride levels in humans, rats, and goats.

In addition, The decreases in blood cholesterol and triglyceride concentrations can be attributed to the increased conversion of cholesterol to bile salts and fecal excretion in the liver due to fenugreek seed's saponin content (Ribes et al., 1986).

Shah and Mir (2004) report that the supplementation of fenugreek seed in the diet of dairy cattle increases the unsaturated fatty acid concentration in milk. Similarly, in the present study, it was determined that the unsaturated fatty acid concentration in milk was increased with the supplementation of fenugreek seed in the goats' diet. The fatty acids stored in ruminant adipose tissue are mainly palmitic, stearic, and oleic acids. If the animal is in a negative-energy-balance state, the stearic acid and oleic acid concentrations in milk increase (Martinez-Marin et al., 2011). Nevertheless, the goats that were used in this study did not lose body weight throughout the experiment. The increase in the

milk oleic acid concentration in the FG treatment is probably associated with the higher supply of stearic acid, and oleic acid due to the fenugreek seed supplementation (Table 1) might have also increased due to the duodenal flow of stearic acid (C:18) and oleic acid into the mammary gland (Loor et al., 2004). Furthermore, Bernard et al. (2005) reported that higher oleic acid concentrations were observed in milk when an animal's diet was supplemented with oleic acid. On the contrary, the fenugreek seed used in our study was richer in linoleic (C18:2n6c) acid (40.88 g per 100 g FA), linolenic (C18:3n3) acid (19.28 g per 100 g FA), and oleic acid (17.69 g per 100 g FA). However, milk linoleic acid and the linolenic acid concentration did not increase with fenugreek supplementation in our study. This may be due to the fact that these fatty acids are mostly biohydrogenated in the rumen (Chillard and Ferray, 2004). Because of the higher biohydrogenation rate of linoleic acid in the rumen, the ratio of oleic acid, which is an isomer of linoleic acid, might have increased in goat milk. The *trans* elaidic acid (t9-18:1) concentration in goat milk was increased with fenugreek seed supplementation. It has been reported that t6, t7, and t8-18:1 fatty acids are derived from the biohydrogenation of oleic acid (Abu-Ghazaleh et al., 2005). Therefore the increase in *trans* elaidic acid in the milk of FG goats could have originate from the microbial activity of rumen bacteria on oleic acid (Bernard et al., 2009). The myristic acid concentration in the milk of the FG treatment animals was lower than that of

CON treatment animals. This reduction is related to the lower *de novo* medium-chain fatty acid synthesis in the mammary gland and may also be related to the higher rate of *trans* fatty acid produced in the rumen, which may have restricted the synthesis of medium-chain fatty acids (Chillard et al., 2001). The increase in milk arachidonic acid levels in the FG treatment may be due to endogenous synthesis, similar to conjugated linoleic acid (CLA) synthesis reported by Griinari et al. (2000). Fenugreek seed is widely employed for its galactogenic activity. However, Korman et al. (2001) and Mazza et al. (2002) have argued that the use of fenugreek seed is restricted due to the fact that its strong, unpleasant odor passes through milk, skin, and urine. It is reported that sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) causes the unpleasant odor associated with fenugreek seed (Blank et al., 1997; Prodebrad et al., 1999). According to Blank et al. (1997), eugenol, linalool, diacetyl, acetic acid, caproic acid, isovaleric acid, and butanoic acid are the flavor components found in fenugreek. Mazza et al. (2002) identified 71 compounds in fenugreek, whereas Mebazza et al. (2009) identified 67 compounds. Differences have also been determined between the volatile compounds in fenugreek seed grown in Sicily, Tunisia, and Turkey (Blank et al., 1997). These differences are probably due to environmental and cultivation conditions or may vary depending on the method used to determine the volatile compounds. In our study, 24 compounds were identified from Turkish fenugreek seed (Table 5), and no sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) (responsible for the unpleasant odor) (Blank et al., 1997; Prodebrad et al., 1999) was detected in the milk samples. The substance with the highest concentration found in the fenugreek seed was (*E*)-2-methyl-2-butenal (7.67 %).

Dietary factors may change the fatty acid composition, nutritional characteristics, and flavor of milk (Chillard et al., 2003). Toso et al. (2002) reported that 2-propanone (acetone), 2-butanone, 2,3-butanedione, acetaldehyde, ethanol, and other such compounds in milk were affected by the feeding regime (pasture or TMR) in cows. In this study, fenugreek seed supplementation increased 2-propanone, 2-butanone, cymene, and eucalyptol concentrations in the milk. Marsili (2003) also reported that the acetone and 2-butanone components of milk originate from the feed. As can be seen from Table 5, 2-propanone, 2-butanone, cymene, and eucalyptol compounds were also determined in the fenugreek seed consumed by the study goats. The differences between the milk volatile compound profile of the CON and FG treatments in this study could be attributed to the fenugreek seed supplementation.

## 5 Conclusions

Fenugreek seed supplementation did not change the milk yield of the goats during the mid-lactation period, but it increased the milk fat concentration and milk fat yield. Fenugreek seed supplementation also enhanced milk quality characteristics in terms of the polyunsaturated fatty acid concentration. The volatile compounds of milk were affected by fenugreek seed supplementation. More studies are required in order to determine the effects of fenugreek seed supplementation on the organoleptic profile, product quality, and consumer preferences of goat milk.

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**Data availability.** The data are available from the corresponding author upon request.

**Author contributions.** HIA and TS designed the study. HIA collected the data. HIA and YKY performed the laboratory analysis. TS and HIA produced the statistical data obtained from the study. HIA wrote the manuscript, and TS and YKY reviewed and edited the manuscript.

**Competing interests.** The contact author has declared that none of the authors has any competing interests.

**Ethical statement.** The protocol for animal use and implementation was confirmed by the ethical guidelines in force at the University of Çanakkale Onsekiz Mart (protocol no. 2017/02-10).

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