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Rodolpho Martin Do Prado,
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Federal University of Rio Grande do Sul,
Brazil
Ana Schogor,
Santa Catarina State University, Brazil

*CORRESPONDENCE

Hassan Rafiee
✉ harafiee@yahoo.com

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Effects of fennel (*Foeniculum vulgare*) seed powder addition during early lactation on performance, milk fatty acid profile, and rumen fermentation parameters of Holstein cows

Erfaneh Moosavi-Zadeh¹, Amin Rahimi², Hassan Rafiee^{3*},
Hamidreza Saberipour⁴ and Ramin Bahadoran⁵

¹Department of Animal Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran,

²Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran,

³Animal Science Research Department, Isfahan Agriculture and Natural Resources Research and Education Center, Agriculture Research, Education and Extension Organization (AREEO), Isfahan, Iran,

⁴Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, ⁵Bahadoran Laboratory of Animal Nutrition and Feed Science, Isfahan, Iran

Introduction: Aromatic and herbal plants usage as feed additives have become a new tendency in dairy cows' nutrition to enhance animal performance. This experiment was performed to study the effects of supplementing fennel seed powder (FSP) to diets during early lactation on performance, milk fatty acid (FA) profile, and rumen fermentation of Holstein dairy cows.

Methods: Twenty-four primiparous Holstein dairy cows (10 ± 3 d in milk, 30 ± 2.1 Kg of milk/d, 610 ± 31 Kg body weight; mean \pm SE) were balanced for actual milk yield and calving date ($n = 8$ per treatment) in a complete randomized design. Animals were allocated randomly to diets containing 0 g/d (0FSP), 25 g/d (25FSP), or 50 g/d (50FSP) FSP, individually top-dressed over the total mixed ration. The experimental period was 45 d consisting of the first 15 d for adaptation and the final 30 d for data collection and sampling.

Results and discussion: Dry matter intake responded quadratically to FSP feeding, and cows fed 25FP treatment had greater DMI than 0FSP treatment. The average ruminal pH value decreased linearly as FSP increased in diets. Ruminal valerate and isovalerate proportion increased linearly as FSP inclusion in diets increased, while acetate proportion decreased and acetate:propionate ratio tended to decrease linearly. Increasing FSP in diets linearly increased serum glucose, globulin, and total protein concentrations. Milk yield increased linearly as FSP inclusion in diets increased, whereas milk composition was unaffected. Increasing FSP in diets linearly increased de novo and mixed FA and decreased preformed FA in milk.

Dietary treatments did not affect saturated FA, whereas unsaturated FA, mono and poly unsaturated FA linearly decreased with increasing FSP inclusion in diets. Moreover, the content of C18:0 tended to decrease, and C18:1 *cis*-9 decreased linearly as FSP inclusion increased. Also, increasing the FSP level in diets decreased linearly non-esterified fatty acids and acetone concentrations in the milk. It could be concluded that FSP addition at 50 g/d could enhance performance of early lactating cows.

KEYWORDS

essential oils, fennel seed, herbal additives, early lactation cow, negative energy balance

Introduction

Cows experience negative nutrient balances after calving for several weeks because dry matter intake (DMI) is less than needed to support milk yield. This imbalance between nutrient intake and demand in early lactation leads to negative energy balance (NEB) and energy mobilization from body fat tissue (Hashemzadeh-Cigari et al., 2015). Mobilization of adipose tissue during NEB increased concentration of non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) during early lactation, which is associated with metabolic and immune dysfunctions such as ketosis and lipodosis. Therefore, dietary strategies should be considered to optimize the metabolism rate of lactating cows during the early lactation period.

Feed additives are used in dairy cow nutrition for different reasons, including covering needs from essential nutrients, increasing feed intake, optimizing feed utilization, and improving overall performance. Phytochemicals, as bioactive plant compounds, have been used as feed additives to promote productivity and prevent diseases in human and animal nutrition (Rochfort et al., 2008). The beneficial effects of phytochemicals on animals' performance may be associated with their positive effect on intake (Saeedi et al., 2017) and nutrient digestion (Mahmoud et al., 2020; Fahim et al., 2021) or their characteristics as antibacterial, antioxidative, and immunostimulation (Hashemzadeh et al., 2022).

Fennel (*Foeniculum vulgare*) is an aromatic plant belonging to the Apiaceae family. Limonene, anethole, α -pinene, fenchone, estragole, and fenchol are the main phytoconstituents of fennel (Hajalizadeh et al., 2019). Because of these essential oil and bioactive compounds, fennel has aromatic (due to essential oils such as fenchone, *trans*-anethole, estragole, and limonene), antioxidant and anti-inflammatory (due to flavonoids, essential oils, phenolic acids, hydroxycinnamic acids, coumarin, and tannin), estrogenic (due to photoanethole and dianethole), antimicrobial (due to 1,3-benzenediol, oleic acid, undecanal, linoleic acid, 5-hydroxy-furanocoumarin, and 2,4-undecadienal) properties (Badgajar et al., 2014; Kargar et al., 2021; Nowroozinia et al., 2022). The fennel antioxidants inhibit the gastrointestinal tract contractions induced by acetylcholine and histamine (Badgajar et al., 2014; Kooti et al., 2015). Also, essential oils in fennel decrease gas production in the gastrointestinal by regulating motility of intestinal smooth muscle (Kooti et al., 2015).

Moreover, it was reported in humans that fennel has positive effects on some metabolism-related disorders, including regulating the appetite and improving hyperlipidemia, mainly by influencing the expression of insulin and leptin receptors (Zakernezhad et al., 2021). Also, estrogens are crucial hormonal regulators of systemic energy homeostasis via regulating adipose tissue metabolism. Estrogens seem to display beneficial actions on insulin and glucose metabolism through the suppression of adipose lipolysis resulting in decreased circulating free FAs levels and the modulation of expression and/or secretion of adipocytokines for the improvement of insulin sensitivity (Kim et al., 2014). Furthermore, Samadi-Noshahr et al. (2021) reported that FSP had hypolipidemic effects *via* its antioxidant properties and ability to stimulate insulin secretion. These results indicate that FSP may prevent body fat mobilization in fresh cows by increasing insulin sensitivity and reducing lipolysis.

Mahmoud et al. (2020) observed improved milk yield with fennel seed powder (FSP) addition at 0.7% of the diet of crossbreed lactating cows for 90 days. Furthermore, FSP supplementation increased yield of milk and energy-corrected milk (ECM) by 10.5 and 16%, respectively, in early lactating buffaloes (Fahim et al., 2021). Also, Attari et al. (2018) evaluated the effects of supplementing dairy cows (30 days in milk) with a photogenic mixture containing FSP at 0.03% of body weight for 63 days. They reported that FSP addition increased actual and component-corrected milk yield without affecting milk composition. Saeedi et al. (2017) observed that supplementation of FSP to the diet of Holstein dairy calves for two weeks increased average daily gain and decreased the age of weaning. Furthermore, Hajalizadeh et al. (2019) stated that FSP addition at 1.5% of diet to growing lambs for 80 days increased DMI and final body weight.

van Haelst et al. (2008) reported that the mobilization of adipose tissue happens earlier than the development of ketosis in the early lactation cows and mobilized FAs are transferred into milk fat; hence, milk long-chain FAs profile change may be a valuable tool for early diagnosis of hyperketonemia. Also, Churakov et al. (2021) indicated that milk FAs could be used to address NEB in early lactation cows and concentrations of C18:0 and C18:1 *cis*-9 are the best variables for early detection of cows with severe NEB. Moreover, it was reported that supplementation of FSP affected milk fatty acid (FA) profile. Mahmoud et al. (2020) reported increased omega-3, polyunsaturated FAs, and conjugated linoleic acid (CLA), while decreased saturated FAs proportions in lactating cows received fennel at 0.7% of diet. For these reasons, milk FAs profile was measured in the current

experiment to investigate the effect of FSP on the energy balance of early lactation dairy cows.

To our knowledge, no data are available on the effects of FSP on performance, milk FAs profile, rumen fermentation, and blood parameters of early lactating cows. We hypothesized that feeding FSP would improve performance without increasing body fat mobilization in dairy cows. Therefore, the objective of the present study was to investigate the impact of increasing levels of FSP as feed additives on DMI, milk yield and FAs profile, rumen fermentation, and some blood parameters of dairy cows during early lactation.

Materials and methods

All animal procedures were approved by the Animal Care and Use Committee of Isfahan University of Technology (IUT, Iran; IACUC #2019/B15) as set by the [Iranian Council of Animal Care \(1995\)](#). The experiment was performed from January to March 2022,

TABLE 1 Chemical components based on the total essential oil (%) distilled from fennel (*Foeniculum vulgare*) seed (n = 3)..

Compound	RI ¹	%
(E)-Anethole (<i>trans</i> -anethole)	1,286	74.5
Fenchone	1,088	9.9
Limonene	1,030	5.8
Estragole (methyl chavicol)	1,197	4.2
α -Pinene	933	0.9
(Z)- β -Ocimene	1,035	0.9
Carvone	1,243	0.4
Myrcene	990	0.53
<i>p</i> -Anisaldehyde, dimethyl acetal	1,254	0.31
1,8-Cineole	1,031	0.6
Sabinene	974	0.34
Camphor	1,144	0.22
(Z)-Anethole (<i>cis</i> -anethole)	1,247	0.21
α -Phellandrene	1,006	0.16
Camphene	951	0.16
γ -Terpinene	1,058	0.22
<i>p</i> -Cymene	1,025	0.17
Germacrene D	1,482	0.15
exo-Fenchyl acetate	1,235	0.12
β -Pinene	978	0.09
allo-Ocimene	1,127	0.1
Carvacrol	1,298	0.1
Terpinen-4-ol	1,172	0.07
Others	—	0.3
Unknown	—	0.1

¹Retention index relative to n-alkanes on column.

at the Saberi poor Animal Husbandry Farm, Nazar Abad, Karaj, Iran (35.903°N, 50.663°E).

Animals and treatments

Twenty-four primiparous Holstein dairy cows (10 ± 3 days in milk, 30 ± 2.1 kg of milk/d, 610 ± 31 kg of body weight) were blocked by actual milk yield and calving date (n = 8 per treatment) in a complete randomized design. Cows were housed in individual pens (4 × 4 m) with concrete floors cleaned regularly and fed a total mixed ration (TMR) ad libitum intake. Dietary treatments were as follows: 1- diet containing 0 g of FSP (0FSP); 2- diet containing 25 g FSP (25FSP); 3- diet containing 50 g of FSP (50FSP). The FSP dosage was chosen from previous studies ([Kargar et al., 2021](#); [Nowroozinia et al., 2022](#)), in which 1.5 and 3 g/d FSP (0.041 and 0.082 g FSP/d per kg BW; mean calf's BW of 36.3 kg) significantly improved the average daily gain and health indicators in dairy calves. Accordingly, we used 0.041 and 0.082 g FSP/d per kg of cow BW, corresponding to 25 and 50 g/d of FSP per cow, with the mean cow's BW of 610 kg.

Chemical components of FSP were measured according to the method described by [Ansari et al. \(2022\)](#). Briefly, FSP (30 g; n = 3) was subjected to hydrodistillation in 1,000 mL of distilled water for 3 h using a Clevenger-type apparatus (HeatingMantle; Sci FineTech Co.). After dehydration over anhydrous sodium sulfate (CAS #7757-82-6), the percentage of oil was calculated. The prepared oil was stored in sealed vials in the dark at 4°C and then subjected to GC and GC-MS analyses to identify of constituents ([Ansari et al., 2022](#); [Table 1](#)).

The forage-to-concentrate ratio of the basal diet was 39:61 on a DM basis, with alfalfa hay and corn silage as the forage sources. Before feeding, alfalfa hay was chopped with a theoretical length of 30 mm using a harvesting machine with a screen size regulator (model NPC260, Niroupayeh Co., Karaj, Iran). Corn silage was used from the silo and sampled weekly for DM content, and its inclusion in the TMR has been adjusted accordingly. Corn and barely grains were ground using a hammer mill with 2 and 3 mm screen sizes, respectively (model AHM80/55, Avartin Co., Tehran, Iran). The TMR was prepared daily and offered twice at 08:00 and 16:00 in an equal amount to ensure 5 to 10% feed refusals. Ingredients and chemical composition of diets are shown in [Table 2](#). Diets were formulated to meet or exceed nutrient requirements stipulated by the [NRC \(2001\)](#). Fennel seed powder was dispersed into 200 g of ground corn grain (as-fed basis) and top-dressed twice daily by mixing into the top portion of the TMR at each feeding. Before feeding, FSP was ground to pass through a 1-mm screen in a Wiley mill (Ogawa Seiki Co. Ltd., Tokyo, Japan). Experimental period was 45 d and cows were adapted to experimental diets during the first 15 d of the trial and the last 30 d for sampling and data collection. Cows had free access to fresh water and diets and were milked thrice daily.

Sampling, measurements, and analyses

Feed intake: The amounts of TMR offered was weighed daily during the sampling period for each cow, and orts were recorded daily every morning before feeding. The TMR and orts samples were taken daily and dried at 68°C for 48 h in a forced-air oven for DMI calculation. Feed intake was recorded by subtracting the total

TABLE 2 Ingredients and chemical composition of the experimental diet.

Items	% on dry matter basis
Ingredients	
Alfalfa hay	16.80
Corn silage	22.40
Beet pulp	4.65
Corn grain	15.08
Barley grain	16.75
Wheat bran	1.68
Soybean meal	9.2
Cottonseed	2.90
Corn gluten meal	0.86
Fat powder ^a	1.90
Meat meal	5.03
Calcium carbonate	0.30
Sodium bicarbonate	1.0
Dicalcium phosphate	0.19
Bentonite	0.26
Toxin binder	0.07
Magnesium oxide	0.26
Vitamin-mineral premix ^b	0.56
Salt	0.11
Chemical composition	
Crude protein	16.71
Ether extract	4.90
Ash	7.80
Neutral detergent fiber	32.70
Non-fiber carbohydrates ^c	39
alcium ^d	1.36
Phosphorus ^d	0.64
Net energy for lactation ^d , Mcal/kg	1.63
^a Palmac 80-16, Erafeed, Malaysia. Composition: moisture 0.5%, crude fat 99.5% (C14:0, 3%; C16:0, 85%; C18:0, 2%; C18:1, 7-10%; C18:2, 2%). ^b Premix contained: kg of mineral-protein supplement containing 1600000 IU of Vitamin A, 250 thousand IU of Vitamin D, 7000 IU of Vitamin E, 250 mg of Biotin, 3 g of Monensin, 16% Calcium, 4% Phosphor, 3% Sulphur, 5% Magnesium, 10 g of Manganese, 14 g of Zinc, 1 g of Iron, 6 g of Copper, 100 mg of Selenium, 200 mg of Iodine, 80 mg of Cobalt. ^c Nonfibrous carbohydrates: 100 - (CP + NDF + ether extract + ash) (NRC, 2001). ^d Calculated from NRC (2001).	

amount of TMR offered daily from that of refusals. Moreover, dried TMR samples were ground to pass a 1-mm screen in a mill (Ogaw Seiki CO., Ltd., Tokyo, Japan) and analyzed (AOAC, 2002) for DM (method 925.40), CP (method 2001.11), ether extract (method 920.39), and ash (method 942.05). Neutral detergent fiber (NDF) content was determined with the methods described by Van Soest et al. (1991) using heat stable α -amylase.

Rumen parameters: Rumen fluid was sampled on day 37 of study at 3 h after the morning feed. Rumen content collecting was performed through the rumen using a custom-designed stomach pump and a tube at one end into a 500 mL container as described by Geishauser (1993). The first 200 mL of fluid pumped out was discarded, and the subsequent was filtered with 4 layers of cheesecloth and immediately after filtration pH was analyzed with a portable pH meter (HI 8318; Hanna Instruments, Cluj-Napoca, Romania). After that, two ruminal fluid subsamples were taken and mixed with chilled 25% (wt/vol) metaphosphoric acid (H_3PO_4) and stored at $-20^\circ C$ for later determination of volatile fatty acids (VFA) and NH_3-N (Heidari et al., 2022). After thawing, the colorimetric phenol-hypochlorite method was used to determine NH_3-N concentration (Broderick and Kang, 1980). Also and for VFA analysis, another rumen subsample was thawed and centrifuged at $10,000 \times g$ at $4^\circ C$ for 20 min and analyzed using gas chromatography ($0.25 \times 0.32, 0.3 \mu m$ i.d. fused silica capillary, model No. CP-9002 Vulcanusweg 259 a.m., Chrompack, Delft, the Netherlands), as described by Bal et al. (2000).

Blood parameters: Blood samples were collected from the coccygeal vein on days 20 and 40 into heparinized tubes (7 mL per sample; Vacutainer, Becton Dickinson, Franklin Lakes, NJ) 3 h after the morning feeding and then immediately placed in iced water. The samples were centrifuged at 3000 rpm for 15 min. The obtained plasma was aliquoted in 1.5-mL microtubes and stored at $-20^\circ C$ until further analyses. After experimental period, plasma samples were thawed and concentrations of glucose (Kit No. 93008), total protein (Kit No. 9304), cholesterol (Kit No. 2118), albumin (Kit No. 9307), and triglyceride (Kit No. 2109) were determined via an automatic analyzer (Alcyon-300 Auto analyzer; DRG Instruments GmbH, Marburg, Germany) using commercially available kits (Pars Azmoon, Tehran, Iran). Serum globulin was determined by subtracting albumin from total plasma protein (Ansari et al., 2022).

Milk yield and composition: Milk yield was recorded thrice daily at 0900, 1700, and 0100 h. Yields were recorded in the last 30 d of the experimental period and averaged to determine the daily mean milk yield. Milk was sampled on d 41 until 45 of study, and samples were preserved with potassium dichromate and held at $4^\circ C$ pending analysis. Milk samples were submitted to Ideh Sazan Rojan Alvand Co. (Alborz, Iran) for fat, true protein, lactose, solids not-fat (SNF), total solids (TS), milk urea nitrogen (MUN), NEFA, BHBA, and FAs analyses using Fourier-transform mid-infrared spectroscopy (FTIR) of CombiScope FTIR 600 HP (Delta Instruments, Drachten, The Netherlands). This method could be helpful for evaluating the energy status and NEB in early lactating cows (McParland and Berry, 2016; Daneshvar et al., 2021). Milk composition for each day was calculated from the composite of each milking corrected for milk yield.

Yields of 3.5% fat corrected milk (FCM, kg/d) and energy corrected milk (ECM, kg/d) were calculated according to the following equations as described by Dairy Records Management Systems (2014):

$$3.5\% \text{ FCM} = (0.432 \times \text{kg of milk yield}) + (16.218 \times \text{kg of fat yield}) .$$

$$\text{ECM} = (12.95 \times \text{kg of fat yield}) + (7.2 \times \text{kg of protein yield}) + (0.327 \times \text{kg of milk yield}) .$$

Feed efficiency (FE) was calculated as actual and component-corrected milk yield (kg/d) divided by DMI (kg/d).

Statistical analysis

Data were analyzed as a complete randomized design using the mixed model procedure of SAS (2002). The statistical model applied included the effects of treatment as a fixed effect and cow within treatment as a random effect, and time (day) entered the model as a repeated statement. Treatments were balanced for actual milk yield and calving date. The following model was used: $Y_{ijk} = \mu + T_i + D_j + (TD)_{ij} + C(T)_{ik} + E_{ijk}$, where Y is the dependent variable; T_i is the fixed effect of treatment ($i = 1, 2, 3$); D_j is the fixed effect of time ($j = 1, 2, 3, \dots, 30$); $(TD)_{ij}$ is the interaction effect between time and treatment; $C(T)_{ik}$ is the random effect of cow within treatment; and E_{ijk} is the residual error. The data were checked for normality and where necessary, suitable Box-Cox transformations were identified using the TRANSREG procedure to normalize the distribution of a particular dataset. The different variance-covariance matrices (Huynh-Feldt (HF), compound symmetry (CS), Toeplitz (TOEP), and first-order autoregressive) were tested and the lowest corrected Akaike's information criterion (AIC) was used for choosing the best matrix. Linear and quadratic contrasts were tested. Significance was declared at $P < 0.05$, and trends at $P < 0.10$.

Results

Dry matter intake and rumen fermentation parameters are indicated in Table 3. The DMI responded quadratically to FSP feeding ($P = 0.040$), and cows fed 25FSP treatment had greater DMI than 0FSP treatment. Ruminant pH decreased linearly as FSP increased in diets, and the average value for cows fed 0FSP, 25FSP, and 50FSP diets were 7.05, 6.86, and 6.79, respectively ($P = 0.044$). No

differences in ruminal $\text{NH}_3\text{-N}$ and total VFA concentrations occurred among treatments. Valerate ($P = 0.001$) and isovalerate ($P = 0.012$) proportion increased linearly as FSP inclusion in diets increased, while acetate proportion decreased ($P = 0.022$) and acetate:propionate ratio tended to decrease ($P = 0.072$) linearly.

Increasing FSP in diets linearly increased plasma glucose ($P = 0.008$), globulin ($P = 0.005$), and TP ($P = 0.08$) concentrations, whereas plasma cholesterol ($P = 0.53$) and triglyceride ($P = 0.15$) concentrations were not affected (Table 4).

As shown in Table 5, milk yield increased linearly as FSP inclusion in diets increased. Increasing FSP in diets linearly increased yields of milk fat ($P = 0.017$), protein ($P = 0.028$), lactose ($P = 0.014$), and TS ($P = 0.008$), whereas milk concentrations of fat, protein, lactose, and TS were not affected. Treatments had no effects on MUN concentration which averaged 12.4 mg/dL. There was a quadratic effect for FE in cows fed increasing amounts of FSP ($P < 0.001$), with cows fed 25FSP having lower FE compared with cows fed 50FSP ($P < 0.001$).

Increasing FSP in diets linearly increased *de novo* ($P < 0.001$), mixed ($P = 0.064$), and decreased preformed ($P = 0.002$) milk FAs, whereas free FAs were not affected by FSP inclusion (Table 6). Milk saturated FAs were not affected by dietary treatments, whereas unsaturated FAs ($P = 0.001$), mono unsaturated FAs ($P < 0.001$), and poly unsaturated FAs ($P = 0.032$) linearly decreased with increasing FSP inclusion in diets. Moreover, content of C18:0 tended to decrease and C18:1 *cis*-9 decreased in milk as FSP inclusion increased linearly. Also, increasing FSP levels in diets decreased NEFA ($P = 0.008$) and acetone ($P = 0.023$) concentrations in milk linearly.

Discussion

In contrast to our finding, FSP supplementation did not affect feed intake in early lactating Egyptian buffaloes (Fahim et al., 2021), whereas

TABLE 3 Dry matter intake and rumen fermentation parameters of early lactating Holstein dairy cows ($n = 8$ per treatment) fed a basal diet supplemented with fennel seed powder (FSP).

Items	Treatments ^a			SEM	P-value	
	0FSP	25FSP	50FSP		Linear	Quadratic
Dry matter intake (kg/d)	19.341 ^b	20.871 ^a	19.665 ^{ab}	0.512	0.660	0.040
Rumen fermentation parameters						
pH	7.051 ^a	6.867 ^{ab}	6.793 ^b	0.084	0.044	0.602
Total volatile fatty acids, mM	69.162	71.162	87.875	7.939	0.110	0.457
Acetate, mol/100 mol	55.756 ^a	53.810 ^{ab}	50.395 ^b	1.535	0.022	0.700
Propionate, mol/100 mol	22.596	23.826	25.077	1.379	0.217	0.995
Butyrate, mol/100 mol	17.795	18.068	19.137	0.841	0.271	0.703
Isovalerate, mol/100 mol	3.166 ^b	3.416 ^b	4.291 ^a	0.292	0.012	0.393
Valerate, mol/100 mol	0.686 ^b	0.877 ^b	1.098 ^a	0.067	0.001	0.858
Acetate/Propionate ratio	2.553	2.333	2.064	0.182	0.072	0.914
$\text{NH}_3\text{-N}$, mg/dL	9.160	10.691	9.388	1.009	0.874	0.264

^a0FSP, 0 g fennel seed powder; 25FSP, 25 g fennel seed powder; 50FSP, 50 g fennel seed powder. ^{a, b} Means within a row with different superscripts are significantly different ($P < 0.05$).

TABLE 4 Blood parameters of early lactating Holstein dairy cows (n = 8 per treatment) fed a basal diet supplemented with fennel seed powder (FSP).

Items	Treatments ^a			SEM	P-value	
	0FSP	25FSP	50FSP		Linear	Quadratic
Glucose, mg/dL	45.28 ^b	51.29 ^{ab}	54.28 ^b	2.192	0.008	0.578
Cholesterol, mg/dL	154.79	162.05	165.71	12.111	0.530	0.904
Triglyceride, mg/dL	46.733	46.812	53.255	3.140	0.156	0.417
Albumin, g/dL	3.152	3.131	3.006	0.075	0.185	0.584
Globulin, g/dL	3.271 ^b	3.449 ^b	3.810 ^a	0.122	0.005	0.548
Albumin/Globulin ratio	1.024 ^b	1.014 ^b	0.851 ^a	0.055	0.038	0.272
Total protein, g/dL	6.424 ^b	6.580 ^{ab}	6.817 ^a	0.095	0.008	0.733

^a0FSP, 0 g fennel seed powder, 25FSP, 25 g fennel seed powder; 50FSP, 50 g fennel seed powder. ^{a, b} Means within a row with different superscripts are significantly different (P < 0.05).

it increased DMI in dairy calves (Fahim et al., 2021; Kargar et al., 2021), lamb (Hajalizadeh et al., 2019), and Mohabadi dairy goats (Yari et al., 2018). Fennel seed powder contains anethole, limonene, α -pinene, fenchol, fenchone, and estragole (Hajalizadeh et al., 2019) and can stimulate the appetite due to both aroma and flavor agents (Kargar et al., 2021), resulting that diet being more palatable and therefore increasing DMI. It was reported that DMI was increased by a flavoring

diet (Saeedi et al., 2017; Kargar et al., 2021). Moreover, FSP improved the digestibility of nutrients (Mahmoud et al., 2020; Fahim et al., 2021), and it could increase DMI due to lower rumen fill (Allen, 2014). It is difficult to explain inconsistent results on DMI. The nutrient composition of the experimental diets, kind and stage of animal, and FSP dosage across different studies were different. For example, early lactation dairy cows were used in the current experiment compared

TABLE 5 Milk yield and composition of early lactating Holstein dairy cows (n = 8 per treatment) fed a basal diet supplemented with fennel seed powder (FSP).

Items	Treatments ^a			SEM	P-value	
	0FSP	25FSP	50FSP		Linear	Quadratic
Yield, kg/d						
Milk	32.266 ^b	33.855 ^a	34.355 ^a	0.409	0.001	0.289
ECM	32.525 ^b	32.500 ^b	36.377 ^a	0.828	0.003	0.068
FCM	33.175 ^b	33.103 ^b	37.298 ^a	0.957	0.006	0.083
Fat	1.140 ^b	1.134 ^b	1.340 ^a	0.054	0.017	0.128
True protein	0.878 ^b	0.868 ^b	0.959 ^a	0.024	0.028	0.110
Lactose	1.540 ^b	1.553 ^b	1.631 ^a	0.024	0.014	0.279
Total solids	3.965 ^b	3.968 ^b	4.340 ^a	0.091	0.008	0.116
Solids not-fat	2.768 ^b	2.815 ^b	2.987 ^a	0.041	0.001	0.236
Milk composition, %						
Fat	3.341	3.324	3.675	0.157	0.149	0.349
True protein	2.715	2.718	2.771	0.044	0.385	0.642
Lactose	4.504	4.548	4.498	0.031	0.882	0.229
Total solids	11.674	11.655	11.936	0.242	0.453	0.619
Solids not-fat	8.145	8.237	8.260	0.059	0.185	0.639
MUN, mg/dL	11.914	12.547	12.619	0.436	0.266	0.604
Efficiency, kg/kg						
Milk/DMI	1.742 ^a	1.601 ^b	1.775 ^a	0.026	0.394	<0.001
FCM/DMI	1.697 ^a	1.556 ^b	1.815 ^a	0.047	0.090	0.002
ECM/DMI	1.664 ^a	1.527 ^b	1.773 ^a	0.040	0.071	0.001

^a0FSP, 0 g fennel seed powder, 25FSP, 25 g fennel seed powder; 50FSP, 50 g fennel seed powder. ^{a, b} Means within a row with different superscripts are significantly different (P < 0.05).

TABLE 6 Fatty acids profile (g/100 g total fatty acids) in milk of early lactating Holstein dairy cows (n = 8 per treatment) fed a basal diet supplemented with fennel seed powder (FSP).

Items	Treatments ^a			SEM	P-value	
	0FSP	25FSP	50FSP		Linear	Quadratic
De novo ^b	26.777 ^b	27.355 ^b	28.971 ^a	0.361	<0.001	0.253
Mixed ^b	29.168	29.305	30.701	0.555	0.064	0.365
Preformed ^b	44.053 ^b	43.338 ^b	40.344 ^a	0.774	0.002	0.242
Free fatty acids	1.475	1.346	1.365	0.083	0.360	0.476
Saturated fatty acids	69.296	69.128	68.759	2.121	0.859	0.969
Unsaturated fatty acids	27.000 ^b	25.203 ^{ab}	22.790 ^a	0.840	0.001	0.767
Mono unsaturated fatty acids	23.940 ^b	22.346 ^b	20.250 ^a	0.667	<0.001	0.761
Poly unsaturated fatty acids	3.284 ^b	3.084 ^{ab}	2.712 ^a	0.176	0.032	0.694
C:16	27.306	27.209	27.725	0.795	0.713	0.756
C:18	17.275	16.903	15.496	0.653	0.067	0.524
C18:1 cis-9	20.159 ^b	18.993 ^b	16.865 ^a	0.475	<0.001	0.417
Non esterified fatty acids, meq/L	494.90 ^b	466.21 ^b	392.91 ^a	24.923	0.008	0.473
BHBA, mmol/L	0.098	0.085	0.102	0.007	0.669	0.094
Acetone, mmol/L	0.229 ^b	0.198 ^{ab}	0.190 ^a	0.011	0.023	0.408

^a0FSP, 0 g fennel seed powder, 25FSP, 25 g fennel seed powder; 50FSP, 50 g fennel seed powder.

^bDe novo FA are defined as FA with chain length <16 carbons (originated from mammary synthesis), preformed FA are defined as FA with chain length >16 carbons (originated from blood), and mixed FA (originated from both sources, C16:0 plus C16:1 cis-9 and iso-C16:0). ^{a, b} Means within a row with different superscripts are significantly different (P < 0.05).

with mid-lactation dairy cows and early lactation buffaloes used by Mahmoud et al. (2020) and Fahim et al. (2021), respectively. Moreover, those experiments (Mahmoud et al., 2020; Fahim et al., 2021) used only one level of FSP in contrast to the present experiment, which used two levels of FSP. The quadratic response of DMI to increasing levels of FSP indicates that various levels of FSP had different effects on intake and performance of dairy cows, and it needs further research to find the best dosage of FSP.

Lower ruminal pH in cows fed FSP may be explained by lower acetate concentration and acetate:propionate ratio. By increasing the digestibility and rate of fermentation (Mahmoud et al., 2020; Fahim et al., 2021), fermentation byproducts production such as total and individual VFAs will increase, leading to a ruminal pH decrease (Church, 1988). In agreement with our finding, calves fed a diet with 0.8% FSP decreased ruminal pH compared to a control group (Saeedi et al., 2017). Moreover, Busquet et al. (2006) indicated that cows supplemented with plant extracts had lower ruminal pH than in control, which showed plant extracts' impact on production of ruminal VFA. In contrast, Santos et al. (2015) in dairy calves and Yari et al. (2018) in Mohabadi dairy goats observed no significant effects on rumen pH by supplementing essential oils.

Feeding diets containing FSP did not affect ruminal NH₃-N concentration. According to Santos et al. (2015) and Saeedi et al. (2017), ruminal NH₃-N concentration increased when plant extracts were added to dairy calves. However, feeding FSP to Mohabadi dairy goats decreased rumen NH₃-N concentration (Yari et al., 2018). Benchaar et al. (2006) showed that rumen NH₃-N concentration and protein digestibility did not change by supplementing a mixture of essential oils (0.75 and 2 g/d) in dairy cows. Moreover, Castillejos

et al. (2007) indicated that feeding a blend of different essential oils, including limonin and thymol, had no effect on rumen NH₃-N concentration but increased total VFA production. They suggested that supplementing essential oils for a long time may cause ruminal micro-organisms adaptation and become resistant to the effects of ingredients in essential oils.

In agreement with our finding, FSP addition decreased acetate proportion and acetate:propionate ratio and increased propionate and total VFA production in Mohabadi dairy goats (Yari et al., 2018) and dairy calves (Saeedi et al., 2017). Furthermore, essential oils supplementation increased the relative abundance of propionate and valerate, whereas it decreased acetate levels in beef cattle (Zhang et al., 2021). Essential oils can interact with microbial cell membranes and inhibit the growth of some gram-positive and gram-negative bacteria. As a result of such inhibition, the addition of some plant extracts to the rumen results in an inhibition of deamination and methanogenesis, resulting in lower acetate and higher propionate concentrations (Calsamiglia et al., 2007). The lower acetate levels in the rumen denote less methane because methanogens could use acetate to produce methane (Cobellis et al., 2016), and the low acetate:propionate ratio indicates a high energy efficiency in feed (Liu et al., 2020). On the other hand, it has been reported that the effects of pure herbal essential oils on rumen fermentation related to ruminal pH (Cardozo et al., 2005), essential oil level (Benchaar et al., 2006), and basal diet (Calsamiglia et al., 2007). Finding the optimum composition and level will prepare an optimum situation for rumen fermentation (Calsamiglia et al., 2007).

Plasma protein represents the total amount of protein present in the blood. It also reflects the two major groups of proteins, i.e.,

albumin and globulin. The increase in TP is due to greater plasma globulin concentration in FSP-fed cows in the current experiment. Also, Mahmoud et al. (2020) reported that the increase in blood TP may be due to the increase in protein digestibility in FSP-fed animals reported by Mahmoud et al. (2020) and Fahim et al. (2021). The same result was observed by Galbat et al. (2014), that the serum TP concentration was increased by adding some medicinal plants to the ration of dairy goats.

Plasma globulin increment with phytogetic addition may be evidence of their merit to increase the immunity status of animals due to inhibiting the growth of pathogens or decreasing their toxins in the gastrointestinal ecosystem (Windisch et al., 2008). Also, Bhatt (2015) indicated that most phytoGENICS have anti-inflammatory, antibacterial, antiviral, anthelmintic, antioxidant, or coccidiostats properties, which may improve the immune system. Results are agreed with those reported by Mahmoud et al. (2020) and Lakhani et al. (2019), who showed enhanced plasma TP and globulin concentrations when FSP was fed to dairy cows and calves, respectively. In disagreement with our finding, Fahim et al. (2021) reported that FSP had no effect on plasma TP and globulin concentration of buffaloes. The linear increase of plasma globulin level with the addition of FSP caused a linear decrease in the ratio of albumin to globulin.

The increase in plasma glucose concentration probably is related to the increase in DM and OM digestibility reported by other studies (Mahmoud et al., 2020; Fahim et al., 2021). In contrast to our finding, plasma glucose levels did not change in buffaloes (Fahim et al., 2021) or decreased in dairy calves (Lakhani et al., 2019) when the FSP was added to diets. These variations between experiments might be due to variations in experimental design, doses supplemented, diets, species, and physiological stage of experimental animals.

Similar to our findings, FSP supplementation increased milk and ECM yield in early lactating buffaloes (Fahim et al., 2021) and crossbred lactating cows (Mahmoud et al., 2020). Also, Attari et al. (2018) observed that FSP supplementation at 0.03% of body weight for 63 days in dairy cows increased milk yield without affecting milk composition. In the current experiment, feed efficiency increased in 50FSP treatment due to increased milk yield without affecting feed intake with supplementations of FSP, in agreement with Fahim et al. (2021).

Milk yield increase may result from improved total tract nutrient digestibility, as mentioned by Fahim et al. (2021) and Mahmoud et al. (2020). Moreover, Windisch et al. (2008) indicated that herbal additives have an improved effect on the productivity and health status of the animal by keeping the digestive tract stability through the prevention of the pathogens' growth and decreasing animal's exposure to toxins. Furthermore, it can suppose that milk yield improvement is a result of increasing rumen VFA concentration with FSP supplementation in the current experiment.

Milk fat content increased by FSP addition in other studies (Mahmoud et al., 2020; Fahim et al., 2021). Despite a linear decrease in rumen acetate production, FSP addition did not affect milk fat content in the present study. Differences in the results may be related to the species of animal, dose of supplements, basal diets, or physiological stage. Many factors affect milk fat content, such as genetics, stage of lactation, concentrate intake, amount and composition of dietary fat, protein and energy intake, and seasonal

effects. Energy intake influences milk fat composition in several ways. In a negative energy balance, dietary supply of glucose decrease, causing lower synthesis of short-chain FAs by mammary tissue and increased mobilization of adipose tissue FAs. This short-chain FAs decrease probably leads to lower milk fat (Palmquist et al., 1993). In our experiment, FSP-fed cows had lower ruminal acetate proportion but higher *de novo* and mixed FAs in milk, and this shows that the increase in milk *de novo* and mixed FAs levels despite the decrease in ruminal acetate level could maintain the milk fat. The current data showed insignificant effect of FSP on milk composition. These results are consistent with those found by Mahmoud et al. (2020); Fahim et al. (2021), and Attari et al. (2018), who reported that FSP had no significant effect on milk composition.

In a meta-analysis study, Daning et al. (2021) reported that using essential oils at a specific dosage has significant effects on milk and FCM yield, and feed efficiency. However, it does not affect the milk composition such as protein, lactose, fat, and MUN.

Supplementation with FSP affected milk FAs profile. Mahmoud et al. (2020) observed increased omega-3, polyunsaturated FAs, and CLA, while decreased saturated FAs proportions in lactating cows received FSP at 0.7% of diet. Furthermore, FSP addition increased proportions *cis*-9, *trans*-11 C18:2, proportion *trans*-10, *cis*-12 C18:2, and total CLA; however, it decreased polyunsaturated FAs without affecting the concentration of C16:0 in early lactating buffalo (Fahim et al., 2021).

In the present study, milk FAs profile and ketone bodies were evaluated by the FTIR analysis. This method could be useful for evaluating the energy status of cows (McParland and Berry, 2016), especially in early lactating period (Daneshvar et al., 2021). Lower preformed and higher *de novo* and mixed FAs in milk of FSP-fed cows might be associated with less BW mobilization than 0FSP cows. Preformed FAs originated from diet and body fat mobilization. As a result, Palmquist et al. (1993) reported that high amounts of preformed FAs in mammary glands inhibit the production of *de novo* FAs. Furthermore, concentration of milk preformed FAs was higher than in mid-lactation dairy cows, as reported by Rico et al. (2014). These findings may be the consequence of greater body fat mobilization and NEB of early lactation cows included in the current experiment, as a similar scenario had been reported by Daneshvar et al. (2021). Also, estrogens are crucial hormonal regulators of systemic energy homeostasis via regulating adipose tissue metabolism. Estrogens seem to display beneficial actions on insulin and glucose metabolism through the suppression of adipose lipolysis resulting in decreased circulating free FAs levels and the modulation of expression and/or secretion of adipocytokines for the improvement of insulin sensitivity (Kim et al., 2014). Furthermore, Samadi-Noshahr et al. (2021) reported that FSP had hypolipidemic effects *via* its antioxidant properties and ability to stimulate insulin secretion. These results indicate that FSP may prevent body fat mobilization in fresh cows by increasing insulin sensitivity and reducing lipolysis.

Gross et al. (2011) demonstrated that long-chain FAs concentrations in milk, especially unsaturated FAs, C18:1 *cis*-9, and C18:0 could be used as indicators for evaluating energy status in dairy cows. Moreover, Churakov et al. (2021) indicated that milk FAs could be used as a tool to address NEB in early lactation cows, and concentrations of C18:0 and C18:1 *cis*-9 are the best variables for early detection of cows with severe NEB. Also, van Haelst et al. (2008)

reported that the mobilization of adipose tissue happens sooner than the ketosis development in early lactation cows, and mobilized FAs are transferred into milk fat; hence, milk long-chain FAs profile change may be a useful tool for primary diagnosis of ketosis. In the current experiment, FSP addition decreased milk content of unsaturated FAs, C18:1 *cis*-9, and C18:0 compared with 0FSP diet, which is consistent with the lower milk acetone and NEFA. It is argued that FTIR predictions for acetone and NEFA are valuable for screening cows on subclinical ketosis, especially when used in combination with other indicators, and can serve in the evaluation of the herd health status with respect to subclinical ketosis (de Roos et al., 2007; Bach et al., 2019). In agreement with our findings, de Roos et al. (2007) and Bach et al. (2019) showed that cows with higher body fat mobilization had higher milk ketone bodies and lower *de novo* FAs, which are promising indicators of subsequent disease or removal in early lactation. Data from the current study indicated that adding FSP increased concentrations of *de novo* and mixed FAs, while concentrations of performed and unsaturated FAs decreased. These results, together, indicate that FSP-fed cows had a lower body fat mobilization, and FSP may improve metabolic statements in early lactation cows.

Supplementing 50 g FSP to early lactating Holstein dairy cows increased milk production and plasma glucose and globulin and reduced body fat mobilization indicated by lower milk long-chain FAs; therefore, FSP addition is proposed as an economically affordable strategy to improve performance and health outcomes.

Conclusions

Adding 50 g of FSP increased milk yield in early lactating Holstein cows without any negative effect on feed intake. Supplementations did not influence milk composition, including fat, protein, lactose, and TS. Moreover, FSP supplementation increased concentrations of *de novo* and mixed FAs and decreased preformed FAs in the milk. Also, unsaturated FAs linearly decreased with increasing FSP inclusion in the diets. Furthermore, increasing the FSP level in the diets decreased the NEFA and acetone concentrations linearly in milk. The ruminal pH value and acetate proportion decreased linearly as FSP increased in diets. Increasing FSP in the diets linearly increased plasma glucose, globulin, and TP concentrations, indicating the positive effect of FSP addition on animal health. The results of this study showed that addition of FSP could enhance the performance of early lactating cows. Also, the results of the milk FAs profile and ketone bodies explained that FSP has a positive effect on body fat mobilization and

could decrease the risk of ketosis in early lactating cows. In agreement with our hypothesis, FSP feeding improved performance of early lactating Holstein cows without increasing body fat mobilization. Further investigations are essential to understand better how FSP impacts digestibility, blood parameters, and body condition score change in transition dairy cows (pre and postpartum).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All animal procedures were conducted with protocols approved by the Animal Care and Use Committee of the Iranian Council of Animal Care (1995).

Author contributions

All authors have made a substantial direct and intellectual contribution to the work ranging from inception of the project idea, funding, project implementation and management, data collection and analysis, writing, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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