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Fennel seed powder supplementation in dairy goat diet: effects on milk productive traits, fatty acid profile and gene expression

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The aim of the present study was to evaluate the effect of dietary supplementation with fennel seeds powder (FSP) on dairy goats. Twenty goats, homogeneous in terms of body weight (BW), days in milk (DIM), daily milk yield (DMY), and parity, were randomly assigned into two groups (CON: control; FEN: fennel). Both groups were fed alfalfa hay and corn in the barn, with the treated group (FEN) receiving an additional 15 g/head/day of FSP. From findings, no significant differences were detected in milk yield or milk chemical composition, although some fatty acids showed variations between the groups. Additionally, a gene expression analysis was conducted on candidate genes related to milk production, lipid metabolism and the antioxidant system without revealing any differences. However, this field of research appears promising, and further studies are needed to standardize the optimal dosage to achieve positive effects on yield and genomic activation.

KEYWORDS

fennel seeds, milk production, fatty acid profile, gene expression, dairy goats

1 Introduction

To improve production efficiency, optimize animal health and address sustainability concerns, researchers and farmers are increasingly turning to nutraceuticals. These bioactive compounds derived from natural sources offer potential health benefits (Losacco et al., 2025). They serve as alternative feed additives and positively influence the immune system through antioxidant, anti-inflammatory and antimicrobial effects (Iommelli et al., 2025a) having as a result improved animal health and productivity.

Fennel (*Foeniculum vulgare* Mill) is an aromatic plant belonging to the Apiaceae family, traditionally used in human health care for its medicinal properties (Badgujar et al., 2014). It is valued for its carminative, digestive and diuretic properties (Rather et al., 2016). Its major bioactive components include phenolic compounds, glycosides and volatile organic compounds (VOCs), with trans-anethole, estragole, limonene and fenchone being the most abundant.

In particular, fennel's galactagogue effect is linked to its high content of trans-anethole, a monoterpene that inhibits dopamine binding to its receptors, acting as an antagonist (Hassanzadeh et al., 2022). This reduces the effect of dopamine on prolactin (PRL) (Benker et al., 1990), allowing PRL to remain active for longer, which directly stimulates milk production by acting on the mammary glands (Lacasse et al., 2012). Although animal studies are scarce, fennel seeds have been tested in dairy ruminants with positive results. In a study, El-Hendawy et al. (2018) tested fennel seed supplementation in Damascus goats and observed improvements in reproductive parameters, milk yield (MY) and milk composition as also observed in Murciana grazing goats in our previous trial (Iommelli et al., 2025b). Recently, Moosavi-Zadeh et al. (2023) investigated the effect of fennel on the diet of dairy cows by assessing milk production characteristics. In both cows and goats, fennel supplementation increased MY, although lactose, protein and fat content showed variable results.

It has already been demonstrated that nutritional factors, including various dietary components such as macronutrients, micronutrients, phytochemicals, bioactive compounds and xenobiotics are able to influence gene regulation (Bonet and Palou, 2020). The increasing use of molecular biology techniques and bioinformatics in research on ruminant nutrition and physiology has significantly deepened our understanding of the regulatory mechanisms underlying key biological processes such as milk production. For example, PUFA have been observed to suppress the expression of the *FASN* (Simopoulos, 2020) and to exert an influence on *SCD* expression (Bernard et al., 2009), both genes involved into the regulatory mechanism of milk fat production.

Therefore, the aim of this study was to evaluate the effects of supplementing the diet of lactating goats with fennel seed powder (FSP). The study also hypothesized that the inclusion of FSP in the diet would increase milk production due to the galactagogue properties of fennel and improve nutritional quality due to its high antioxidant content. In addition, gene expression analyses were carried out to identify differences in the expression of genes involved in milk production, antioxidant activity and lipid metabolism.

2 Material and methods

2.1 Experimental design

The feeding trial was performed at Azienda Zootecnica Antonio Amato located in Casaletto Spartano (SA, Italy; 832 m a.s.l., 40°09' N;

15°37'E) on 20 multiparous lactating Murciana goats according to the Animal Welfare and Good Clinical Practice (Directive 2010/63/EU), lasted 4 weeks and was approved by the local Animal Ethic Committee (protocol number: PG/2019/0070006). Twenty animals, homogeneous for parity (3rd), days in milk (DIM: 60 ± 7.2), body weight (BW: $50 \text{ kg} \pm 2.0 \text{ kg}$) and MY ($1922 \pm 210 \text{ g/day}$), were randomly allocated into two groups (CON: control and FEN: fennel). All the animals were housed in stall and received 1 kg of alfalfa hay and 700 g/head/day of corn meal in individual pen; in addition, group FEN received 15 g/head/day of fennel seeds powder (FSP, 400–450 μm) (*Foeniculum vulgare*) which was mixed into the concentrate before been offered to the animals. An adaptation period of 7 days was considered for the treated group for the acclimatization to the new dietary treatment. The dose was selected according to previous studies in which fennel seeds were used and were able to exert significant positive results on milk productive traits (Iommelli et al., 2025b; El-Hendawy et al., 2018). All the animals had free access to fresh water and feed refusal were daily measured.

2.2 Samples collection

Feed samples were collected at the beginning and at the end of the trial whereas individual MY was daily measured and samples were collected consecutively for the three days at the beginning and at the end of the trial.

2.3 Total phenolic content and antioxidant activity

The total phenolic content (TPC) in fennel seeds samples was determined according to the Folin-Ciocalteu colorimetric method described by Taga et al. (1984). A 0.5 g of powder was dissolved in 10 mL of distilled water and vortexed at 2000 rpm for 5 min (Heathrow Scientific Vortexer, Vernon Hill, IL, USA). The supernatant was immediately collected in a Falcon tube. An aliquot of 250 μL supernatant was mixed with 1250 μL Folin-Ciocalteu reagent and 1000 μL of 7.5% anhydrous sodium carbonate. The mixture was vortexed and incubated in the dark for 120 min. Absorbance was then measured at 760 nm using a UV-VIS spectrophotometer (Hitachi U-2001, Tokyo, Japan). A calibration curve was constructed with gallic acid solutions (0–1000 $\mu\text{g/mL}$), and results were expressed as mg gallic acid equivalents (GAE)/g. To evaluate the antioxidant activity of the samples, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method was used according to Re et al. (1999), with modifications. Specifically, a 7 mM solution of ABTS in 2.45 mM aqueous potassium persulfate was prepared, and after 16 h of incubation at room temperature in the dark, the solution was diluted with ethanol to obtain an absorbance of 1.000 ± 0.020 at 734 nm. After, 100 μL of the extract according to Wojdyło et al. (2007), was added to 1000 μL of this solution. Measurements of absorbance were taken at 734 nm after 2.5 min of incubation (Hitachi U-2001, Tokyo, Japan). In addition, Trolox was used as the reference and activities were expressed as Trolox equivalents (mg/g TE).

The antioxidant activity of the feed was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sanchez-Moreno et al., 1998). Briefly, 0.1 mL of sample was added to 3.9 mL of DPPH solution (60 μ M in methanol) and incubated in the dark for 45 min. Absorbance was measured at 515 nm after 30 min of incubation in the dark at room temperature using a Hitachi U-2001 spectrophotometer (Hitachi, Tokyo, Japan). A calibration curve was prepared with DPPH solutions ranging from 10 to 60 μ M. The half-maximal effective concentration (EC₅₀, μ g/mL) was defined as the sample concentration required to reduce the initial DPPH absorbance by 50%.

2.4 Volatile organic compounds analysis

The VOCs extraction from fennel seeds samples was performed by solid-phase microextraction (SPME), whereas the GC-MS analysis was carried out with a gas chromatograph (Perkin Elmer, Waltham, MA, USA) coupled with a mass spectrometer (SQ8S; Perkin Elmer, USA). The gas chromatograph was equipped with an Elite-5MS column (Perkin Elmer, USA). A total of 5 g of sample were mixed with 10 mL of saturated sodium chloride solution (360 g/L), and then 10 μ L of internal standard solution (4-methyl-2-heptanone; 10 mg/kg in ethanol) was added. The vials were sealed with a polytetrafluoroethylene-silicone septum (Supelco, Bellefonte, PA, USA) and stirred at 60°C; VOCs were extracted from the headspace with a divinylbenzene-carboxen-polydimethylsiloxane SPME fiber (Supelco, USA) with an exposition time of 60 min. After adsorption time, the extracted VOC were thermally desorbed into the gas chromatograph injector splitless mode for 1 min at 250°C. The oven temperature was held at 50°C for 1 min, increased at a rate of 3°C/min up to 200°C and held for 1 min, and then increased from 200°C to 250°C at 100.15°C/min and held for 15 min. Helium was used as a carrier gas at a flow rate of 1 mL/min. The mass spectrometer operated in electronic impact ionization mode at 70 eV, and data were collected in full scan mode. Source and interface temperature were held at 250°C. Compounds were identified by comparing their mass spectra with those contained in the National Institute of Standards and Technology (NIST) 14 library (Gaithersburg, MD, USA). The compounds were considered as correctly identified when their spectra presented a library match factor > 85. The VOCs were expressed on percentage of total volatile compounds detected.

2.5 Chemical composition

2.5.1 Feed

Corn meal, fennel seeds and alfalfa hay were analyzed for dry matter (DM), crude protein (CP), ash, crude fiber (CF) and ether extract (EE) contents, according to AOAC (2012) procedures, and structural carbohydrates were determined as suggested by Van Soest et al. (1991). Feed nutritional value was calculated according to Inra-Nozière et al. (2018).

2.5.2 Milk

Milk samples (150 mL) were analyzed for fat, lactose and protein content by Milko Scan 133B (Foss Matic, Hillerød, Denmark) standardized for goat milk.

2.6 Fatty acids analysis

2.6.1 Feed

Lipid fraction of feed samples was extracted from 15 g of sample using a mixture of chloroform and methanol (2/1 vol/vol) according to Gray et al. (1967) for the analysis of fatty acid (FA) profile. Lipid extract was methylated adding 1 mL of hexane and 0.05 mL of 2 N methanolic KOH. Separation of the methyl esters in forage and cheese samples was performed according to Di Trana et al. (2015). Fatty acid methyl esters (FAME) were identified with reference to the retention time of FA standard mixture of Supelco 37 Component FAME Mix (Supelco, Bellefonte, PA). The content of FA was quantified using internal standards (Supelco, Bellefonte, PA) added during the methylation step. Briefly, 100 mg of lipid extract was mixed with 50 μ L of 2 N methanolic KOH and 1 mL of hexane containing the internal standards (20 mg/mL) according to Giorgio et al. (2019). Fatty acid methyl esters were fractionated over a CP-SIL883 column (100 $\times$ 0.25 mm i.d., film thickness 0.20 μ m fused silica; Varian, Palo Alto, CA, USA) in a Shimadzu (Model 2GC17A) gas chromatograph with a Hewlett-Packard HP 6890 gas system and using flame ionization detection. Helium was used as carrier gas at a constant flow rate of 1.7 mL/min. The oven temperature was programmed as follows: 175°C, held for 4 min; 175–250°C at 3°C/min; and then maintained for 20 min. The injector port and detector temperature were 250°C. Samples (1 μ L) were injected with an auto-sampler. Output signals were identified and quantified from the retention times and peak areas of known calibration standards. Composition was expressed as percentages of the total FA. All determinations were carried out in triplicate.

2.6.2 Milk

The FA profile (50 mL of milk) was determined as described by Tudisco et al. (2014). Briefly, milk fat was extracted by using hexane and isopropane (3/2 v/v) (Hara and Radin, 1978). The FAME were prepared by direct transesterification with sulfuric acid and methanol (1:9, v/v) of the lipids (Christie, 1993) and then analyzed in a gas chromatograph (Agilent technologies, model 5890) fitted with an SP-2560 fused silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μ m film thickness, Supelco, Inc., Bellefonte, PA, USA). Helium was used as carrier gas and set at a constant pressure of 180 kPa, splitting flow of 50 mL/min, and injection volume of 1 μ L. The column parameters were: initial temperature of the column maintained at 170°C for 15 min; then, with an increase of 5°C/min, it was brought up to 240°C. The total execution time was 64 min. Fatty acid peaks were identified using pure methyl ester external standards (Larodan Fine Chemicals, AB, Limhamnsgårdens Malmö, Sweden). Additional standards for CLA isomers were obtained from Larodan. Chromatogram peak areas were acquired and calculated by Chemstation software (Agilent,

technologies) and expressed as g/100g, considering 100g as the sum of the areas of all FAME identified. All determinations were carried out in triplicate. Additionally, FA ratios C14:1/C14:0, C16:1/C16:0, C18:1/C18:0 were measured to evaluate the desaturation activity of stearoyl CoA desaturase (Tudisco et al., 2019a).

2.7 Gene expression analysis

2.7.1 Milk fat sampling and RNA isolation

At two time points (T0 = beginning of the trial and T1 = end of the trial), during the morning milking, the milk samples for gene expression analysis were collected as follows. The udder was washed, dried and disinfected and a total of 50 mL of milk, obtained from the morning milking, were individually collected from each animal in sterilized, RNase free falcon tubes and immediately centrifuged at 4000 g × 10 minutes at 4°C. One gram of milk fat layer was then scooped out in 15 mL tubes with 3 mL of TRIzol™ LS Reagent (Invitrogen Life Technologies) and vigorously shaken and vortexed for 30 seconds. The samples were immediately transferred in dry ice, transported to the lab and kept at -80°C until further analysis. The RNA extraction was performed following TRIzol™ LS Reagent protocol: after thawing in ice, the samples were vortexed and centrifuged at 10000 g × 10 minutes at 4°C in order to separate the liquid phase, which was transferred into four 2-mL sterilized tubes and added with 200 µL of chloroform. After centrifugation (10000 g × 15 minutes at 4°C) only the upper phase was taken and added in a new tube with isopropanol (500 µL) and left at -20°C over-night for RNA precipitation. After brief vortexing, the samples were centrifuged at 10000 g × 10 minutes at 4°C to obtain a small pellet by removing gently the isopropanol. One step of ethanol washing was performed by adding 1 mL of ethanol (75%) in each tube and completely removing it after a brief centrifugation (7500 g × 5 minutes at 4°C). The pellets obtained were then dissolved in 40 µL of sterilized RNase free water and the kept at -80°C. Two aliquots of RNA samples were subjected to quantitative and qualitative analysis respectively with NanoDrop (ND-1000 spectrophotometer; NanoDrop, Labtech) to measure the absorbance at 260, 280 and 320 and a 2100 Bioanalyzer (Agilent Technologies) to determine RNA integrity. The mean value of RNA integrity number (RIN) was 4.5 (± 0.8), which is consistent with the results of other studies on milk fat compared to other matrices (Li et al., 2016).

2.7.2 Reverse transcription and real time qPCR

Reverse transcription (RT) was performed from 1 µg of total RNA by using High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems) in a total volume of 20 µL, composed by 6 µL of RNA, 3 µL (2ng/µL) of Luciferase RNA Control (Promega) and 11 µL of Master Mix, according to the manufacturer's instructions. The complementary DNA (cDNA) obtained was then stored at -20°C for RT-qPCR (Real Time qPCR). Samples of cDNA were finally subjected to RT-qPCR in duplicate with MicroAmp™ Fast Optical 96-Well Reaction Plates (Applied Biosystems) using Power SYBR™ Green PCR Master Mix (Applied Biosystems). The candidate genes

(*LPL1*, *NRF2*, *SCD1*, *SOD*, *TLR4*, *PRLR*) were investigated and normalized by adopting Luciferase as reference gene (Jiwaji et al., 2010; Johnson et al., 2005). Each well was filled with 5 µL of cDNA (previously diluted 1:50 with nuclease free water) and 15 µL of Master Mix assembled by 10 µL of Mix SYBR, 2 µL of specific primers (1 µL Forward and 1 µL Reverse) and adjusted to volume of 15 µL with nuclease free water. RT-qPCR reaction was performed using 7300 Real-Time PCR System (Applied Biosystems) set up with the following program: denaturation at 95°C for 10 minutes; 40 cycles of PCR reactions at 95°C for 15 seconds and 60°C for 45 seconds according to the hybridization temperature. The samples were run in duplicate and compared to reference gene to obtain ΔCt (Terzi et al., 2004). Primers were specifically designed on goat reference mRNA sequences and were purchased by Merck (Sigma-Aldrich). Gene primers are reported in Table 1.

2.8 Statistical analysis

Data were analyzed by the one-way ANOVA with SPSS IBM software (V29.0.1.0). Differences were considered statistically significant at $P < 0.05$. Relative mRNA levels were then calculated for each gene using $2^{-\Delta Ct}$ method. The Ct of reference gene was subtracted from Ct of each gene to obtain ΔCt and then relative mRNA value was calculated by the formula $2^{-\Delta Ct} \times 100$. Data were analyzed with two-way ANOVA with SPSS IBM software (V29.0.1.0) for repeated measures considering “treatment” and “period” as fixed factors and “animal” as a random factor.

3 Results

The antioxidant activity, TPC and VOCs composition of FSP are reported in Table 2. In particular, **trans-anethole** was found to be the most abundant VOC, followed by limonene, estragole and fenchone. Results on feed chemical composition are reported in Table 3. The FSP was characterized by a high lipid content. Corn meal and alfalfa hay had the highest amount of polyunsaturated fatty acids (PUFA). Specifically, corn meal was characterized by the highest amount of C18:2n6 (linoleic acid) whereas alfalfa hay had the highest amount of C18:3n3 (linolenic acid). The key FA of FPS were C18:1n12, characteristic of the family Apiaceae and C18:2n6.

In Table 4 MY and chemical composition are presented. No feed refusal was detected; therefore, the dry matter intake (DMI) was 1460 g/head/day. Group FEN had higher MY even if it was not statistically significant ($P > 0.05$). No differences were observed for fat and lactose content whereas protein content resulted significantly lower in the supplemented group ($P < 0.05$). Fat, lactose and protein yield were also measured as g/day and were not affected by the treatment ($P > 0.05$).

Milk FA profile of the two groups is reported in Table 5. Only few differences were detected between groups. Specifically, among short chain FA, C8:0, C10:0 and C12:0 resulted significantly lower ($P < 0.05$) in group treated and regarding long chain FA only C22:6 differed between groups, being higher ($P < 0.05$) in group FEN.

TABLE 1 Primer sequences and conditions used for real-time qPCR.

Gene symbol	Encoded protein	Accession no ¹	Nucleotide sequence (5' – 3') ²	Product size (bp)	Source
<i>LPL1</i>	Lipoprotein Lipase	DQ997818.2	F: TTCAGAGGCTATTACTGGAATCC R: ATGTCAATCACAGCATTCATTCTACT	235	Bernard et al., 2005a
<i>NRF2</i>	Nuclear eritroid factor 2	NM_001314327.1	F: CCAACTACTCCCAGGCAGCCC R: AGCAGTGGCTACCTGAACG	227	Wang et al., 2021
<i>SCD1</i>	Stearoyl-CoA Desaturase	NM_173959	F: TGCTGACAACCTTATCTGGATGC R: AAGGAATCCTGCAAACAGCTA	179	Bernard et al., 2005a
<i>SOD</i>	Super oxide dismutase	AB201469.1	F: AGGCAAAGGGAGATAAAGTCGTCG R: TGCACCTGGTACAGCCTTGTGTATTG	113	He et al., 2012
<i>TLR4</i>	Toll Like Receptor 4	JF825527	F: CTTGCGTCCAGGTTGTTCTCTAA R: CTGGGAACCTGGAGAAGTTATG	153	Paul et al., 2015
<i>PRLR</i>	Prolactin receptor	NM_001039726	F: CATGGATACTGGAGTGAGTGGA R: TGTCTTCACTGGGAAGTCAT	245	Safayi et al., 2010
<i>Luciferase</i>	Luciferase	–	F: AGA GAT ACG CCC TGG TTC CT R: ATA AAT AAC GCG CCC AAC AC	202	Bui et al., 2009

¹Accession number of the sequence used to design primers.

²Sequences: F, forward primer; R, reverse primer.

In Table 6 the main FA classes, ratios and indices are depicted. No differences were detected between groups for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA content whereas *de novo* FA (C4:0 to C16:0) resulted significantly lower in group FEN.

In Table 7 the mRNA levels of the candidate genes for the two groups are presented. The expression of the candidate genes was not influenced by the treatment or by the period of sampling. The mRNA levels were found to be the highest for *SCD1*, followed by *LPL* and *SOD*. In contrast, the lowest values were observed for *PRLR*, *NRF2* and *TLR4*.

4 Discussion

4.1 Diet

The chemical composition of alfalfa hay displayed high crude protein content and nutritive value, as reported by Zicarelli et al. (2022). Additionally, the hay exhibited a considerable concentration

of n3 PUFA compared to corn meal and FSP. Indeed, the inclusion in the diet of n3 PUFAs has been demonstrated to increase the levels of beneficial FA in food of animal origin, thereby exerting favorable effects on human health (Trinchese et al., 2019). The results demonstrated that fennel seeds were rich in lipids, in accordance with the observations of Unal et al. (2013). The fennel FA profile was also found to be characterized by a high content of MUFA, primarily represented by petroselinic acid, and a relatively low content of PUFA and SFA, as reported by Najdoska-Bogdanov et al. (2015). Petroselinic acid (C18:1n12) is a distinctive constituent of the Apiaceae plant family, exhibiting particularly high concentrations in fennel oil. A further distinctive feature of the fennel FA profile is the presence of C11:0, which has been demonstrated to possess antimicrobial activity, particularly against bacteria responsible for alimentary diseases, in the form of a monoacylglycerol preparation (Doležalová et al., 2010).

4.2 Milk yield, chemical composition and fatty acid profile

The efficacy of fennel seeds as a galactagogues feeding supplement in several animal species has already been demonstrated (Moosavi-Zadeh et al., 2023; El-Hendawy et al., 2018; Fahim et al., 2022; Iommelli et al., 2025b) with results showing an improvement of performances in terms of MY and chemical composition. Indeed, fennel seeds are a rich source of trans-anethole, a compound that works as an antagonist of dopamine and is therefore capable of increasing PRL release. Furthermore, it has been demonstrated that fennel seeds improve feed efficiency and feed intake in calves and dairy cows (Kargar et al., 2021; Moosavi-Zadeh et al., 2023). In contrast to the findings of El-Hendawy et al. (2018), in the present work MY wasn't affected by the treatment. Nevertheless, the productive characteristics exhibited by both groups are consistent with those reported for

TABLE 2 Fennel seeds powder antioxidant activity, TPC and VOCs composition.

Item	
ABTS (mg/g TE)	6.65 ± 0.11
DPPH (EC50 µg/ml)	11.58 ± 0.48
TPC (mg GAE/g)	4.03 ± 0.08
VOCs (%)	
Limonene	13.2 ± 0.45
Fenchone	1.9 ± 0.12
Estragole	4.1 ± 0.30
Trans-Anethole	74.8 ± 0.21

TABLE 3 Feed chemical composition (g/kg DM) and fatty acid (FA) profile (g/100g lipid).

Item	Corn meal	Alfalfa hay	FSP
CP	89.81 ± 1.60	189.70 ± 1.19	130.11 ± 0.7
EE	29.72 ± 0.40	18.52 ± 0.30	82.75 ± 0.18
Ash	11.62 ± 0.11	98.61 ± 0.83	13.81 ± 0.81
CF	29.61 ± 0.11	288.86 ± 0.06	111.90 ± 1.50
NDF	–	430.22 ± 0.89	–
ADF	–	350.31 ± 0.25	–
ADL	–	99.01 ± 1.13	–
UFL/kg DM	1.02	0.77	0.80
FA expressed as g/100 g of lipid			
C11:0	nd	nd	5.78 ± 0.02
C12:0	nd	nd	0.01 ± 0.01
C14:0	nd	6.3 ± 0.02	nd
C16:0	12.08 ± 0.12	22.45 ± 0.12	4.22 ± 0.01
C18:0	2.79 ± 0.08	2.65 ± 0.03	2.04 ± 0.02
Σ SFA	14.87 ± 0.12	31.4 ± 0.12	12.87 ± 0.12
C16:1	0.04 ± 0.01	nd	0.20 ± 0.01
C18:1n9	28.03 ± 0.11	6.53 ± 0.05	0.31 ± 0.01
C18:1n12	0.15 ± 0.02	0.87 ± 0.01	76.23 ± 0.15
C20:1n9	nd	nd	0.12 ± 0.01
Σ MUFA	28.07 ± 0.12	6.53 ± 0.02	76.34 ± 0.21
C18:2n6	54.63 ± 0.21	19.56 ± 0.10	9.84 ± 0.05
C18:3n3	2.41 ± 0.02	42.48 ± 0.15	0.25 ± 0.02
C20:5n3	nd	nd	0.04 ± 0.01
C22:6n3	nd	nd	0.02 ± 0.01
Σ PUFA	57.04 ± 0.31	62.04 ± 0.21	10.46 ± 0.15

Murciana goats (Ehsaninia et al., 2025). This breed has become increasingly prominent in European countries with a substantial population, wide distribution and with high-quality milk production (Miranda et al., 2019). Furthermore, this breed is characterized by a notable degree of adaptability, attributable to its capacity for extensive grazing and strong resilience (Kaveh Baghbadorani et al., 2024). In the present trial, lower milk protein content was observed in the group that had been supplemented, which contrasts with the findings of El-Hendawy et al. (2018) on Damiani goats. On the opposite, daily protein yield showed no significant alterations, nevertheless, it exhibited a marginal lower concentration in group F when compared to group C. This outcome is likely attributable to the slightly higher milk yield observed in Group F. Although this result was not statistically significant, it enabled group F to maintain its protein yield at a level comparable to that of group C, despite the lower percentage of protein. With regard to milk FA composition, the group receiving FSP

supplementation exhibited significantly reduced *de novo* FA content, particularly in C8:0 (caprylic), C10:0 (capric), and C12:0 (lauric acid) FA. These FA are naturally present in foodstuffs and are particularly abundant in goat milk (Kim and Rhee, 2016). They have been demonstrated to possess antimicrobial properties, specifically against various foodborne pathogens, including *Campylobacter jejuni*, *Cronobacter* spp., *Helicobacter pylori* and *S. aureus* (Kim and Rhee, 2016). Additionally, capric and caprylic acids are primarily responsible for the distinctive aroma of goat milk (Lo Presti et al., 2023). Petroselinic acid (C:18:1n12) content was found to be similar in the milk of both groups. Research on the metabolism of petroselinic acid remains limited. However, in certain studies incorporating species of Apiaceae into the diet of lactating goats, variations in rumen metabolism have been identified (Kholif et al., 2021; Morsy et al., 2018). This FA has been linked to the rumen metabolism of α -linolenic acid and to mammary desaturation of the resulting isomers (Marín et al., 2015). In addition, rumen biohydrogenation of unsaturated fatty acids suggests a potential modification of dietary petroselinic acid prior to its incorporation into milk fat (Marín et al., 2015). However, the extent to which this modification occurs and its effect on the levels of petroselinic acid in goat's milk require further research. In the present trial, C22:6 (docosahexaenoic acid, DHA) was also affected by the treatment, exhibiting higher levels in group FEN. The mechanisms by which DHA is incorporated into ruminant milk are complex and vary according to several factors, as comprehensively delineated in the review by Huang et al. (2020). As stated by the authors (Huang et al., 2020), the source of DHA in ruminant milk is a direct transfer of this FA from the diet and an alteration of ALA in hepatocytes. In the present experiment, the presence of these two FA in fennel seeds, albeit in small amounts, may have contributed to the increase of DHA in the milk of group F. However, ruminal biohydrogenation processes have been observed to reduce milk DHA content, as it is converted to a C22:6 isomer that is then hydrogenated to C22:5 fatty acid in the rumen. Nevertheless, the enzymes responsible for this process remain to be fully elucidated (Toral et al., 2018). This FA has significant medical implications, as its dietary presence has been linked to the prevention of numerous human afflictions, including cancer and heart disease (Stillwell and Wassall, 2003).

TABLE 4 Daily milk yield (DMY) and chemical composition.

Item	CON	FEN	SEM	P-value
DMY (g)	1553.00	1697.50	131.959	0.598
Fat (%)	4.02	3.76	0.083	0.125
Lactose (%)	4.18	4.25	0.072	0.668
Protein (%)	3.95	3.26	0.108	<0.001
Fat (g/day)	62.25	62.91	4.884	0.948
Lactose (g/day)	64.27	71.61	5.302	0.503
Protein (g/day)	61.21	56.72	5.066	0.670

BW, body weight; DMY, daily milk yield; SEM, standard error of the means.

TABLE 5 Milk FA profile expressed as g/100g of milk fat.

Item	CON	FEN	SEM	P-value
C4:0	2.980	3.178	0.2136	0.658
C6:0	1.677	1.634	0.1057	0.847
C8:0	3.117	2.612	0.0933	0.003
C10:0	12.447	10.372	0.3292	0.000
C11:0	0.348	0.272	0.0220	0.083
C12:0	5.459	4.473	0.1969	0.008
C13:0	0.073	0.060	0.0056	0.266
C14:0	10.967	10.923	0.2209	0.924
C14:1	0.222	0.225	0.0152	0.909
C15:0	0.872	0.847	0.0425	0.771
C16:0	31.161	31.387	0.6322	0.864
C16:1	0.733	0.826	0.0501	0.368
C17:0	0.465	0.398	0.0247	0.184
C17:1	0.054	0.027	0.0139	0.359
C18:1 c6	0.028	0.026	0.0038	0.795
C18:0	8.070	9.463	0.4931	0.164
C18:1 t9	0.403	0.494	0.0307	0.143
C18:1 c9	17.323	18.092	0.4900	0.449
C18:1 c11	0.204	0.227	0.0133	0.406
C18:1 n12	0.041	0.072	0.0215	0.124
C18:2 t6	0.145	0.135	0.0105	0.654
C18:2 c6	1.350	1.548	0.0614	0.109
C20:0	0.229	0.247	0.0103	0.408
C20:1	0.042	0.045	0.0032	0.695
C18:3 n3	0.300	0.341	0.0180	0.262
C18:2c9t11	0.164	0.121	0.0148	0.148
C18:2t10c12	0.144	0.170	0.0143	0.378
C20:2 n6	0.009	0.010	0.0012	0.711
C22:0	0.108	0.118	0.0072	0.487
C22:1	0.017	0.019	0.0014	0.629
C20:4 n6	0.119	0.121	0.0062	0.846
C22:2 n6	0.022	0.013	0.0024	0.051
C24:0	0.040	0.035	0.0034	0.453
C20:5 n3	0.035	0.038	0.0029	0.702
C24:1	0.023	0.034	0.0054	0.312
C22:4 n6	0.010	0.011	0.0027	0.969
C22:5 n3	0.028	0.029	0.0020	0.967
C22:6 n3	0.035	0.052	0.0030	0.002

SEM, standard error of the means.

TABLE 6 Main milk FA classes and ratios.

Item	CON	FEN	SEM	P- value
SFA	78.020	76.025	0.6104	0.104
MUFA	19.053	20.614	0.5541	0.165
PUFA	2.368	2.594	0.0928	0.234
omega 6	1.658	1.841	0.0701	0.202
omega 3	0.400	0.461	0.0190	0.112
CLA	0.309	0.292	0.0110	0.444
PUFA/SFA	0.030	0.034	0.0014	0.191
omega 6/omega 3	4.188	4.031	0.1083	0.487
LA/ALA	4.646	4.661	0.1811	0.969
AA/EPA	3.416	4.811	0.7747	0.384
De novo	38.160	34.591	0.6602	0.004
Mixed	31.891	32.210	0.6356	0.81
Preformed	29.384	32.414	0.9123	0.099
C14:1/C14:0	0.020	0.020	0.0016	0.907
C16:1/C16:0	0.023	0.026	0.0016	0.452
C18:1/C18:0	0.003	0.002	0.0003	0.582

De novo FA, (C4 -C16); Mixed FA, (C16, 0-C16, 1); Preformed FA, (> C16); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acids; LA, linoleic acid; ALA, alpha linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; SEM, standard error of the mean.

4.3 Gene expression

Research has demonstrated that mRNA extracted from MFG exhibits a high degree of similarity to mRNA extracted from MEC during the production of milk. This makes MFG a reliable and accessible source for investigating gene expression related to lactation (Brenaut et al., 2012). Nevertheless, several authors (Li et al., 2016; Nichols et al., 2020) found that mRNA isolated from MFG yielded average low RIN values (< 7), as in the present trial. As proposed by Lemay et al. (2013), the observed low RIN of mRNA extracts are intrinsic to the sample type. This is due to the presence of low molecular weight RNA fragments in the mRNA from MFG, resulting from the presence of small amounts of cytoplasmic material from mammary epithelial cells within the cytoplasmic crescents. Nonetheless, this technique has been demonstrated to enable successful gene expression analysis (Brenaut et al., 2012; Jiménez-Montenegro et al., 2025).

Nutrition is a pivotal environmental determinant in modulating gene–environment interactions. Breakthroughs in molecular biology and genomics have enabled increasingly sophisticated investigations into the role of dietary components in regulating gene expression and influencing phenotypic outcomes. For example, PUFA have been observed to suppress the expression of the FASN (Simopoulos, 2020). In addition to nutrients, non-nutritive dietary phytochemicals, such as phenolic compounds, which are present in FSP, have also being investigated for their

TABLE 7 Candidate gene expression at T0 (at beginning of the trial) and T1 (at the end of the trial).

Target genes	Time	CON	FEN	SEM	P-value		
					G	T	G×T
LPL1	T0	0.1945	0.1875	0.03765	0.758	0.426	0.421
	T1	0.2113	0.1823	0.04531			
NRF2	T0	0.0041	0.0245	0.00581	0.171	0.245	0.124
	T1	0.0058	0.0195	0.00490			
SCD1	T0	0.2985	0.3124	0.06152	0.974	0.345	0.854
	T1	0.3046	0.3098	0.07561			
SOD	T0	0.0894	0.0984	0.03456	0.455	0.124	0.795
	T1	0.0937	0.1233	0.01925			
TLR4	T0	0.0031	0.0315	0.00459	0.140	0.612	0.235
	T1	0.0024	0.0218	0.00652			
PRLR	T0	0.0165	0.0164	0.00451	0.816	0.175	0.328
	T1	0.0142	0.0129	0.00273			

SEM, standard error of the means.

effects on various aspects of animal metabolism. Nevertheless, the regulation of gene expression in response to dietary changes occurs at multiple levels, including transcription (via transcription factors), post-transcription (such as mRNA stability), translation (e.g., initiation or microRNA activity), and post-translation (e.g., enzyme turnover or activity regulation) (Bernard et al., 2013). However, it is frequently unclear whether the regulatory agents are the nutrients themselves, their metabolites, or the hormones produced in response to dietary shifts. Furthermore, the majority of studies are unable to identify the precise level of regulation involved, as measurements of mRNA, protein levels, and enzyme activity are rarely taken concurrently. No significant differences were observed in the expression of lipogenic genes (*LPL1*, *SCD1*) between the experimental groups. In contrast to other lipogenic genes, which are less influenced by dietary factors, *SCD* is typically more responsive to dietary UFA supplementation, particularly in subcutaneous adipose tissue (Bernard et al., 2005a; Iommelli et al., 2021).

The relationship between *SCD* expression and milk quality has been the subject of extensive research. Stearoyl-CoA desaturase is responsible for the production of over 75% of the total CLA in milk (Chilliard et al., 2006). Nutritional factors, particularly those containing lipids, have been demonstrated to exert an influence on *SCD* expression. Tudisco et al. (2019a, 2019b) demonstrated that linseed supplementation reduced *SCD* activity, a result that was supported by Tsiplakou et al. (2009) and Bernard et al. (2009). Nevertheless, the altered activity of *SCD* was not correlated with changes in *SCD* mRNA levels, as observed in the present study. *PRLR* expression levels were examined due to the galactagogue properties of fennel seeds, which are known to influence prolactin (PRL) levels. Despite this, no significant differences in *PRLR* expression were found between the experimental groups. The anti-inflammatory properties of fennel, which are commonly observed in medicinal herbs, have been investigated by examining

the expression of Toll-like receptor 4 (*TLR4*), which activates nuclear factor (NF)- κ B, a key regulator of numerous genes involved in inflammatory responses (Chang et al., 2015). Liu et al. (2015) demonstrated that high-grain feeding influenced *TLR* mRNA levels and altered the bacterial composition of the ruminal epithelium in male goats, thereby establishing a connection between the microbial community and *TLR* expression. The current study revealed no significant differences in *TLR4* mRNA levels. The treatment did not affect the expression of genes involved in antioxidant activity, including *SOD* and *NRF2*. This finding may be attributed to the relatively brief supplementation period. Accordingly, further investigation is required to ascertain the optimal time of administration and dosage in order to evaluate the impact on mRNA levels of candidate genes.

4 Conclusion

The study demonstrates that FSP supplementation did not significantly affect MY, in contrast to previous findings. However, it has been observed that milk protein content is reduced in the supplemented group. Furthermore, the supplementation has been shown to influence milk FA composition, leading to a decrease in *de novo* FA such as caprylic, capric, and lauric acids. These acids play a key role in the antimicrobial properties and aroma of goat milk. An increase in DHA levels was observed in the FSP-supplemented group, which may offer health benefits. In terms of genomic results, no significant changes were detected in the expression of lipogenic genes (*LPL1*, *SCD1*) or prolactin receptor (*PRLR*) gene, despite the known galactagogue properties of fennel seeds. The expression levels of *TLR4*, a gene involved in inflammatory responses, and antioxidant-related genes (*SOD*, *NRF2*) remained unchanged. The absence of significant genomic alterations may be attributed to the

relatively brief supplementation period. Further studies are required to determine the optimal dosage and duration of FSP supplementation, as well as its effects on different lactation stages or primiparous animals, in order to enhance comprehension of its genomic and productive outcomes.

Data availability statement

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

Ethics statement

The animal study was approved by the Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Animal Ethic Committee (protocol number: PG/2019/0070006). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PI: Investigation, Conceptualization, Writing – original draft. VT: Formal Analysis, Writing – review & editing. EC: Writing – review & editing, Formal Analysis. GB: Formal Analysis, Writing – review & editing. FS: Writing – review & editing, Data curation. MF: Investigation, Writing – review & editing. FI: Funding acquisition, Writing – review & editing. RT: Conceptualization, Writing – review & editing, Funding acquisition.

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