The use of ensiled olive cake in the diets of Friesian cows increases beneficial fatty acids in milk and Halloumi cheese and alters the expression of *SREBF1* in adipose tissue

M. C. Neofytou,[1] D. Miltiadou,[1] E. Sfakianaki,[2] C. Constantinou,[1] S. Symeou,[1] D. Sparaggis,[3]
A. L. Hager-Theodorides,[2] and O. Tzamaloukas[1]*
[1]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, PO Box 50329, Cyprus 3036
[2]Department of Animal Science, Agricultural University of Athens, 11855 Athens, Greece
[3]Agricultural Research Institute, Nicosia, PO Box 22016, Cyprus 1516

ABSTRACT

This study aimed to evaluate the effect of dietary inclusion of ensiled olive cake, a by-product of olive oil production, on milk yield and composition and on fatty acid (FA) profile of milk and Halloumi cheese from cows. Furthermore, the effect of olive cake on the expression of selected genes involved in mammary and adipose lipid metabolism was assessed in a subset of animals. A total of 24 dairy cows in mid lactation were allocated into 2 isonitrogenous and isoenergetic feeding treatments, named the control (CON) diet and the olive cake (OC) diet, in which part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled OC as 10% of dry matter according to a 2 × 2 crossover design with two 28-d experimental periods. At the end of the second experimental period, mammary and perirenal adipose tissue samples were collected from 3 animals per group for gene expression analysis by quantitative reverse-transcription PCR. The expression of 11 genes, involved in FA synthesis (*ACACA*, *FASN*, *G6PDH*), FA uptake or translocation (*VLDLR*, *LPL*, *SLC2A1*, *CD36*, *FABP3*), FA saturation (*SCD1*), and transcriptional regulation (*SREBF1*, *PPARG*), was evaluated. No significant differences were observed between groups concerning milk yield, fat percentage, protein percentage, and protein yield (kg/d), whereas milk fat yield (kg/d) increased in the OC group. Dietary supplementation with ensiled OC modified the FA profile of milk and Halloumi cheese produced. There was a significant decrease in the concentration of de novo synthesized FA, saturated FA, and the atherogenic index, whereas long-chain and monounsaturated FA concentration was increased in both milk and cheese. Among individual saturated FA, only stearic acid was elevated, whereas among individual monounsaturated FA, increments of oleic acid (C18:1 *cis*-9) and the sum of C18:1 *trans*-10 and *trans*-11 acids were demonstrated in milk and Halloumi cheese produced. Although no diet effect was reported on total polyunsaturated FA, the concentration of CLA *cis*-9,*trans*-11 was increased in both milk and Halloumi cheese fat of the OC group. The expression of the genes tested was unaffected apart from an observed upregulation of *SREBF1* mRNA expression in perirenal fat from cows fed the OC diet. Milk FA differences observed were not associated with alterations in mammary expression of genes involved in FA synthesis, uptake, translocation, and regulation of lipogenesis. Overall, the inclusion of ensiled OC in cow diets for a 4-wk period improved, beneficially for human health, the lipid profile of bovine milk and Halloumi cheese produced without adversely affecting milk yield and composition or the expression of genes involved in lipid metabolism of mammary and adipose tissues in cows.

Key words: ensiled olive cake, milk fatty acid, Halloumi cheese, gene expression

INTRODUCTION

Milk fatty acid (**FA**) composition has been associated with human health and disease prevention (reviewed in Parodi, 2004; Palmquist, 2009; Ferlay et al., 2017). Inclusion of different UFA in the diet of lactating cows is a reasonable attempt to improve milk fat composition for human consumption (reviewed in Chilliard et al., 2000, 2003; Ferlay et al., 2017). Among all the different sources of unsaturated fats that have been used, the use of agroindustrial by-products could be a cost-effective way of enhancing the quality of milk and its products. Crude olive cake (**OC**), a by-product of olive oil production that is available in appreciable quantities in the Mediterranean basin, represents an interesting

supplement for ruminants as a forage replacement that may improve the lipids of milk (Molina-Alcaide and Yáñez-Ruiz, 2008).

Supplementation with OC has been mainly used in sheep diets (reviewed in Molina-Alcaide and Yáñez-Ruiz, 2008). Feeding trials including dried (Abbeddou et al. 2011a,b, 2015), partly destoned dried (Vargas-Bello-Pérez et al., 2013), or partly destoned fresh (Chiofalo et al., 2004) OC showed decreased SFA and increased MUFA, whereas varying results were obtained for CLA or other linoleic acids in dairy ewe milk (Symeou et al., 2019). A limited number of studies have examined the effect of processed OC on the FA composition of milk or cheese produced in goats (Molina-Alcaide et al., 2010; Arco-Pérez et al., 2017) and cattle (Castellani et al., 2017). Castellani et al. (2017) showed an increase in MUFA and a decrease in SFA and in atherogenic and thrombogenic indices in the milk and cheese of cows fed dried olive pomace.

During the last decade, experiments examining the effect of ruminant diets on FA composition also sought to understand the effects of dietary treatments on adipose tissue and mammary gland lipid metabolism and their contribution to changes in milk FA by looking more specifically at the relationship between milk FA composition and expression of lipogenic genes (Bernard et al., 2017; Toral et al., 2017; Fougère and Bernard, 2019). Among the complex network of genes coordinating milk fat synthesis and secretion, acetyl-CoA-carboxylase (*ACACA*), FA synthase (*FASN*), and glucose 6 phosphate dehydrogenase (*G6PDH*) are key genes encoding enzymes involved in de novo FA synthesis, and very low density lipoprotein receptor (*VLDLR*) in concert with lipoprotein lipase (*LPL*) take up and hydrolyze triacylglycerides (Bionaz and Loor, 2008). Translocators including solute carrier family 2 member 1 (*SLC2A1*) and FA translocator (*FAT/CD36*) play a major role in FA transport, and FA binding protein 3 (*FABP3*) provides substrates to stearoyl-CoA-desaturase 1 (*SCD1*), the most abundant gene in the mammary gland, decreasing the saturation content of FA in ruminant milk (Bionaz and Loor, 2008). Additionally, sterol regulatory element binding transcriptional factor 1 (*SREBF1*) and peroxisome proliferator activated receptor gamma (*PPARG*) constitute regulators of milk lipid metabolism (Osorio et al., 2016).

The inclusion of OC in ruminant feed is quite limited because of its low nutritive value, seasonal availability, and spoilage when stored without preservation for a long time (Hadjipanayiotou, 1999; Owaimer et al., 2004). Despite these disadvantages, OC is characterized by a high oil percentage and high concentration of oleic acid that could improve the milk lipid profile (Chiofalo et al., 2002). Research studies in Cyprus have proposed

an ensiling method as an attempt to overcome the limitations of using fresh or dried OC, allowing the use of this by-product in animal nutrition throughout the year (Hadjipanayiotou, 1999). Recently, we examined the effect of the supplementation of Chios sheep diets with ensiled OC on the FA profile of their products (Symeou et al., 2019). So far, no studies have examined the effect of ensiled OC supplementation on the FA content of bovine milk and Halloumi cheese or the possible effect of any form of OC supplementation on bovine lipogenic gene expression. The objective of the present study was to assess the effects of the addition of ensiled crude OC in the diet of mid-lactation Holstein-Friesian cows on (1) the FA composition of milk and Halloumi cheese and (2) the expression of 11 genes involved in FA synthesis (*ACACA*, *FASN*, *G6PDH*), FA uptake or translocation (*VLDLR*, *LPL*, *SLC2A1*, *CD36*, *FABP3*), FA saturation (*SCD1*), and transcriptional regulation (*SREBF1*, *PPARG*) of lipid metabolism in the mammary and adipose tissues.

MATERIALS AND METHODS

Animals, Management, and Experimental Diets

The experiment was performed in the Agricultural Research Institute (facilities in Athalassa, Nicosia) in Cyprus. All experimental procedures were carried out according to the regulations of the national legislation (Republic of Cyprus, 1994) and international guidelines (European Union, 2010) and approved by the corresponding departmental committee of the Cyprus University of Technology. No animals were killed as the experiment was adjusted to the scheduled program of slaughtering for renewing the herd of the Agricultural Research Institute farm.

A total of 24 Holstein-Friesian mid-lactating cows in their fourth lactation were divided into 6 groups of 4 animals each, homogeneous for age, milk yield (mean ± SEM: 29.31 ± 1.31 kg/head per day), and BW (mean ± SEM: 582.0 ± 11 kg), and allocated randomly to 2 feeding treatments (3 groups per treatment). Animals were used in a replicated 2×2 Latin square to test the effects of the 2 treatments during two 28-d experimental periods. Cows were housed indoors and assigned to 2 isoenergetic and isonitrogenous feeding regimens (Table 1). The control group (**CON**) was fed a conventional diet, whereas the OC group received the conventional diet in which part of the forages were replaced with 5 kg/cow per day of ensiled OC (fresh OC with DM content of 47.7%), obtaining a concentrate:forage ratio of 64:36 in both treatments.

The OC was constituted from a mixture of skins, pulp, woody endocarp, and seeds obtained after extrac-

tion of olive oil from locally cultivated olives (Ladolia and Kalamon cv.). The OC was collected using a 3-stage oil mill and ensiled according to the method developed as follows. After olive oil extraction, crude OC was obtained immediately and accumulated on the surface of the clean, hard floor of the silos. The pile of OC that did not contain any additives or other feed materials for helping the fermentation process was covered with a black polyethylene film (8 mm thick) and firmly closed. The film covering the pile was stretched to expel the air, and soil was used to cover the edges of the film. The ensiled OC was fermented in the silos for 3 to 4 mo before use.

Dry matter intake was monitored daily during the last week of each experimental period. Animals of both groups were fed individually with automatic feeders (Westfalia, Albersdorf, Germany) offering the concentrates 4 times per day, and forages were divided and offered manually to each group after morning (0430 h) and evening (1630 h) milkings. The OC supplement was offered directly after morning milking, and cows consumed it entirely within about 15 min. Feeds were collected at the beginning and at the end of each experimental period and mixed per treatment, and a final sample was taken for analysis. Water was offered ad libitum. The chemical composition of the 2 treatments is presented in Table 1. Dry matter, ash, crude fat, and CP were determined as described by AOAC International (2005). Crude fiber, ADF, ADL, and amylase NDF were measured according to the method of Van Soest et al. (1991).

Measurements and Milk Sampling

All animals were machine milked (Fulwood, Shropshire, UK) twice daily (at 0430 and 1630 h) and milk yields were recorded electronically (AfiMilk MPC Milk Meter, Kibbutz Afikim, Israel) at each milking, but only measurements taken the last week of each experimental period were analyzed statistically. Raw milk samples for the determination of the lipid profile were collected from each cow during the 2 consecutive milkings (morning and evening) at the end of each experimental period (wk 4 and wk 8) and were stored at −80°C for further analysis. Measurements for total fat, protein, and SNF were obtained using combined thermo-optical procedures (Lactostar 3510, Funke Gerber, Berlin, Germany) calibrated previously for protein with the Lowry protein assay, and measurements for fat were obtained with the Gerber method 989.05 (AOAC International, 2005).

At the end of the second experimental period (wk 8), bulk milk obtained from cows of each treatment (CON and OC) was converted into 7 different Halloumi cheeses (14 samples total) according to the method of Papademas and Robinson (1998). Cheese samples were collected (250–300 g) from each barrel, placed in plastic bags, and vacuum-stored at −20°C until analysis.

Lipid Analysis

The milk fat isolation was carried out according to the rapid double centrifugation method of Feng et

Table 1. Ingredients and chemical composition of dietary treatments and the olive cake silage used

Item	Treatment[1] CON	OC	Ensiled OC
Ingredient, %			
OC	—	9.22	
Alfalfa	7.1	7.07	
Barley hay	17.7	13.26	
Barley straw	10.9	6.36	
Concentrate mix[2]	64.3	64.3	
Chemical composition, % of DM unless noted			
DM, %	96.3	95.83	47.74
CP	18.95	18.87	5.32
Crude fat	1.82	2.34	6.88
Crude fiber	17.49	18.89	49.53
Ash	7.35	6.82	2.71
Amylase NDF	35.82	36.54	71.56
ADL	3.8	5.82	28.40
ADF	21.12	22.4	54.93
ME,[3] MJ/kg	9.60	9.38	—

[1]CON = control; OC = part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled olive cake as 10% of DM.

[2]Concentrate mix = 16% barley, 21% maize, 17.8% soybean meal 48%, 13% sunflower cake, 10% wheat bran, 18% sugar beet pulp, and 4.24% mineral and vitamin mix.

[3]Values from NRC (2001).

al. (2004) with slight modifications. Briefly, 20-mL aliquots of fresh milk were first centrifuged at 17,800 $\times$ g for 30 min at 4°C. The resulting fat cakes were removed, placed in new tubes, and allowed to melt at room temperature for 20 min. The samples were then recentrifuged at 19,500 $\times$ g for 20 min at room temperature, and 20-mg aliquots of the resulting lipid cake were removed to fresh tubes and dispersed in 1 mL of *n*-hexane by shaking.

For the feed fat isolation, the method used was as follows. After crude fat determination (Soxtec method), 2 mL of hexane was added to the residue in the aluminum collection pots and transferred to a glass tube (pre-weighed). Then, the residue was dried down under a gentle stream of nitrogen gas, and the glass tube was reweighed. Subsequently, 1 mL of hexane was added for redissolving the residue, and then an aliquot containing 20 to 25 mg of crude fat was removed and put into fresh tubes.

The cheese fat was extracted with diethyl ether as follows: 50 mL of diethyl ether and 0.5 g of Na_2SO_4 was added to 30 to 40 g of cheese samples and mixed vigorously. After 15 min, the mixture was filtered through filter paper (Whatman no. 4, Sigma-Aldrich, St. Louis, MO) from a funnel. The filtrate was centrifuged for 2 min at 2,500 $\times$ g at room temperature to remove the undesired particles that originated from cheese. The liquid phase of diethyl ether and oil was taken into a centrifuge test tube and diethyl ether was removed using a rotary evaporator at 40 $\pm$ 1°C. Then, the sample was flushed with nitrogen to remove the remaining ether from oil.

Fatty acid methyl esters of milk, Halloumi cheese, and feed lipids were prepared by transesterification with methanolic potassium hydroxide according to the ISO (2002) method. Essentially, 40 µL of transesterification reagent (11.2% KOH in methanol) was added to the tubes containing extracted lipids from milk, cheese, and feeds and shaken vigorously for 1 min. The tubes were then allowed to incubate for a further 5 min at room temperature before the addition of 0.1 g of sodium bisulfate. The particulate material was then removed by centrifugation at 350 $\times$ g for 3 min at room temperature, and aliquots of the clear organic phase were transferred to amber gas chromatography vials and stored at -70°C until analyzed. Fatty acid profiles were generated by analyzing the FAME samples on a GCMS-QP2010 Plus gas chromatography mass spectrometer (Shimadzu, Duisburg, Germany) equipped with an HT280T autosampler (HTA, Brescia, Italy) following the method of Kramer et al. (2008). Typically, 1-µL aliquots of FAME were separated with a split ratio of 1:20 using an Agilent CP-Sil 88 fused silica capillary column (100 m $\times$ 0.25 mm i.d., 0.2 µm film thickness). The column was held for 4 min at 70°C after injection, increased at 13°C/min to 175°C, and then held at that temperature for a further 27 min. The temperature was then raised to 215°C at 4°C/min, at which it was held for a further 36 min. Helium was the carrier gas at 1 mL/min, with both injector and interface temperatures of 225°C. Chromatographic profiles were analyzed using Shimadzu GCMS Postrun Solution software, where individual peaks were identified by comparison of their retention indices with those of commercially available authentic standards (Supelco 37-FAME standard mix, *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, C18:1 *trans*-11; Sigma-Aldrich, Gillingham, UK) using the National Institute of Standards and Technology 08 and 21 mass spectral libraries and cross referencing with chromatograms and spectrograms reported in the literature (Kramer et al., 2008; Tsiafoulis et al., 2014). All FAME peaks identified were quantitated by peak integration, and individual FAME were expressed as a percentage of the total fat (Butler et al., 2011).

The atherogenic index was determined in milk and Halloumi cheese using the formula proposed by Ulbricht and Southgate (1991):

$$\text{atherogenic index} = (C12:0 + 4 \times C14:0 + C16:0)/$$
$$(\Sigma\text{MUFA} + \Sigma\text{PUFA}).$$

Desaturation index (DI) was determined in milk using the formula suggested by Garnsworthy et al. (2010):

$$\text{desaturation index} =$$
$$(C14:1 \; cis\text{-}9/C14:0 + C14:1 \; cis\text{-}9) \times 100.$$

Tissue Sampling for Expression Analysis

At the end of the second experimental period (wk 8), 3 cows per treatment, randomly selected from each group, were transferred to an adjacent abattoir immediately after milking, and 50 g of perirenal and 50 g of mammary gland tissues were collected under sterile conditions (12 samples total). All samples were snap-frozen in liquid N_2 immediately and stored at -80°C until RNA extraction.

Primer Design, RNA Extraction, Synthesis of cDNA, and Real-Time PCR

Primer design was performed using the Primer-BLAST tool at the National Center for Biotechnology Information (NCBI; Bethesda, MD) platform using mRNA (preferably reference where available) sequences

for each target or housekeeping gene obtained from the NCBI database (Table 2). Total RNA from perirenal adipose (25 mg) and mammary gland (50 mg) tissue was isolated using NucleoZOL reagent (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's instructions. Briefly, 25 or 50 mg of tissue were homogenized in 500 µL of NucleoZOL reagent. Adipose tissue samples were centrifuged at 12,000 $\times$ g for 5 min at room temperature, and supernatant below the fatty layer was transferred into a new tube. Then, 200 µL of RNase-free water was added, and samples were vortexed and incubated at room temperature for 15 min and then centrifuged at 12,000 $\times$ g for 15 min. Supernatants (500 µL) were mixed with 500 µL of isopropanol, incubated at room temperature for 10 min, and then centrifuged at 12,000 $\times$ g for 10 min at room temperature. Pellets were washed twice with 75% ethanol and centrifuged at 8,000 $\times$ g for 3 min at room temperature. Finally, pellets were air-dried and diluted in 60 µL of RNase free water and samples were stored at −80°C for further analysis. The RNA purity (260/280 and 260/230) and concentration (ng/µL) were assessed using a micro-volume UV spectrophotometer (Quawell Spectrophotometer 3000, Lab Supplies Scientific, San Jose, CA). The cDNA was synthesized from 1 µg of total RNA in 20-µL reactions using the PrimeScript RT-PCR reagent kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions, and samples were stored at −20°C for further analysis.

The mRNA abundance of 11 candidate genes, shown in Table 2, was assessed via quantitative real-time reverse-transcription PCR (**RT-qPCR**). To account for variation in RNA integrity, RNA quantification, and cDNA synthesis, the mRNA abundance was normalized using the geometric mean of 3 reference genes [ubiquitously expressed transcript (*UXT*), ribosomal protein S9 (*RPS9*), and ribosomal protein S15 (*RPS15*)], which have been recommended as the most suitable reference genes in the bovine mammary gland during lactation (Bionaz and Loor, 2007).

The RT-qPCR reactions were performed in an ABI 7500 Real-Time PCR system (Applied Biosystems, Darmstadt, Germany) using FastGene IC Green 2× Universal Mix (Bioline, London, UK). Each 10-µL reaction contained 1.0 µL of cDNA (synthesized from ~12.5 ng of total RNA), 400 nM forward and reverse primer, and 5.0 µL of IC Green Master Mix (Bioline). The RT-qPCR analyses of each studied gene were performed using cDNA from 6 biological replicates, with 3 technical replicates per biological replicate. The qPCR thermal protocol used was as follows: 1 cycle of 95°C for 2 min, 40 cycles of 95°C for 5 s, 60°C for 30 s, and 95°C for 30 s. This was followed by a melt-curve analysis to ensure specific amplification. Real-time PCR runs

with efficiencies between 86 and 110% were considered acceptable and used for data analysis.

Data were analyzed using the 7500 Fast Software option for quantitation-relative standard curve with SYBR green reagents (version 2.3; Applied Biosystems). Threshold cycle ($\mathbf{C_T}$) values were obtained for each reaction using the automatic C_T option in which the software calculates the baseline start and end values and the threshold in the amplification plot for a set of reactions. The software uses the baseline and threshold to calculate the C_T for each reaction. To perform relative quantitation of mRNA levels for each gene, a set of standards, consisting of 5-point serial dilutions (1:1, 1:3, $1:3^2$, $1:3^3$, and $1:3^4$) of cDNA prepared from a pool of cDNA from adipose and mammary tissue, were used along with the samples at every qPCR run. Results from the standards reactions (triplicates for each dilution point) were used to generate the relative standard curve for each gene under study. The corresponding standard curve was identified as the best-fit regression line of C_T (dependent variable, values obtained from the reactions as described above) on the log(Qty) [independent variable with values log(1), log(1/3), $\log(1/3^2)$, $\log(1/3^3)$, and $\log(1/3^4)$] described by the regression line formula $C_T = m [\log (Qty)] + b$, where m is the slope, b is the y-intercept, and Qty is the relative mRNA level of the gene (quantity). Relative mRNA levels for each gene and biological sample were estimated as the mean Qty of 3 technical replicates, obtained using the regression formula mentioned above and applying the C_T values obtained from the reactions as described above.

Statistical Analysis

All data at the animal level from the two 28-d experimental periods were subjected to an analysis of a 2 × 2 AB–BA crossover design (Kaps and Lamberson, 2009) using SAS software (version 9.4, SAS Institute Inc., Cary, NC) to test the effects of the OC feeding treatment. The statistical model included the fixed effects of period, experimental diet, and sequence (the order in which treatments were allocated to each animal). Differences between the diet means were tested based on least squares means difference and declared significant when the P-value was less than 0.05. P-values between 0.05 and 0.10 were interpreted as a trend toward significance.

The FA content in Halloumi cheese and feeds results were subjected to a 2-group t-test with 7 and 3 replications, respectively. The normalized relative mRNA expression data were subjected to nonparametric 1-way ANOVA using SAS Studio software (university edition, version 3.8, SAS Institute Inc.) because data were not normally distributed. For each gene, the dependent

Table 2. Sequences (5' to 3') and efficiencies of the primers used in real-time quantification PCR

Symbol	Name	Primer[1]	Access no.	Amplicon, bp	R^2	Efficiency, %
ACACA	Acetyl-CoA-carboxylase A	F: ATGACGAAGACAAGGTCCCC R: ACCAGGAAAGTAAGGCGTCG	NM_174224.2	169	0.997	100
FASN	Fatty acid synthase	F: CACACTCCATCCTCGCTCTC R: GCCATATAGTCCGGCCTTCC	NM_001012669.1	206	0.996	86
G6PDH	Glucose 6 phosphate dehydrogenase	F: CCATGAGACCTGCATGAGCC R: AGATCCTGTTGGCAAACCTCAG	XM_024987752.1	200	0.996	87
SREBF1	Sterol regulatory element binding transcription factor 1	F: ACATCTCTTGGAGCGGAGCAC R: CTGAGAACTCCTTGTCCCCC	NM_001113302.1	83	0.997	95
VLDLR	Very low density lipoprotein receptor	F: AGAGGACAATGGCCGAGAGT R: GACAGCAAGAGGGGAAGGATG	NM_174489.2	114	0.999	86
LPL	Lipoprotein lipase	F: GGAAAGATCAGAGTAAAGGCAGGA R: CCAAGTTTCAGCCAGACTTTCTATT	NM_001075120.1	150	0.998	86
SLC2A1	Solute carrier family 2 member 1	F: GTCGTGTCGCTATTTGTGGTG R: GCCACAATGCTCAGGTAGGA	NM_174602.2	149	0.991	94
FAT/CD36	Fatty acid translocase	F: ACTGCGGATGGAATTTACAAAG R: ATGAGGCTGCATCTGTACCATTA	NM_001278621.1	142	1	96
FABP3	Fatty acid binding protein	F: AGACCACAGCAGATGACAGG R: TGGGTGAGTGTCAGAATGAGTTT	NM_174313.2	142	0.997	91
PPARG	Peroxisome proliferator activated receptor γ	F: TTGTGAAGGATGCAAGGGTTTCT R: CATGCGCCCAAACCTGATG	NM_181024.2	175	0.998	94
SCD1	Stearoyl-CoA desaturase	F: ACATTGATCCCCACCTGCAA R: CAAAAACGTCATTCTGGAACGC	NM_173959.4	184	0.999	93
Housekeeping gene						
UXT	Ubiquitously expressed prefoldin like chaperone	F: CTCAGCGACAACCTCACCAA R: TGTAGCTCTCTAAGCCCCTCT	NM_001037471.2	80	0.994	99
RPS9	Ribosomal protein S9	F: AGGATTTCTTGGAGAGACGCC R: TGCTTGCGGACCCTGATG	NM_001101152.2	112	0.994	97
RPS15	Ribosomal protein S15	F: GATGGCGGAAGTGGAACAGA R: CTGCATCAGTTGCTCATAGGAC	NM_001024541.2	109	0.991	109

[1]F = forward; R = reverse.

Table 3. Fatty acid composition (expressed as a percentage of total FAME) of the dietary treatments used and ensiled olive cake (OC)

Item	Treatment[1]				Ensiled OC
	CON	OC	SEM	P-value	
C12:0	1.05	0.68	0.09	*	—
C14:0	1.55	0.85	0.19	NS	—
C16:0	26.83	23.35	0.81	**	13.45
C16:1 *cis*-9	0.25	0.57	0.07	**	1.48
C18:0	2.04	2.58	0.12	***	4.15
C18:1 *cis*-9	21.16	33.30	2.73	***	63.58
C18:2n-6	23.52	21.39	0.51	**	13.12
C18:3n-3	2.23	1.68	0.18	NS	1.46

[1]CON = control; OC = part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled olive cake as 10% of DM.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

variable was the relative normalized mRNA expression level (12 biological replicates, 6 per treatment group) and the classification variable was the treatment group (2 treatment groups: control and OC). Differences between treatment means were tested based on Wilcoxon scores followed by Kruskal-Wallis test with df = 1 and were declared significant when $P < 0.05$.

RESULTS

OC Silage and Diets

The FA composition of OC silage and diets is shown in Table 3. The inclusion of OC in the diets had significant effects on most FA. Oleic acid (C18:1 *cis*-9; $P < 0.001$) was the major FA found, at a concentration of around 30% of the dietary lipids of the OC-supplemented diet. Palmitic (C16:0; $P < 0.01$) and linoleic (C18:2n-6; $P < 0.01$) acids were also abundant, whereas stearic (C18:0; $P < 0.001$) and linolenic (C18:3n-3) acids were found in lesser quantities in both treatments. The OC diet showed a significantly lower content of palmitic and linoleic acids compared with the CON diet. In contrast, an increased proportion of stearic acid in the OC diet was demonstrated.

Performance Variables of Cows

Table 4 shows the DMI, milk production and yield, fat and protein yields, and composition of the milk samples after 4 wk of supplementation. Supplementation with OC did not influence the milk and protein yields or the milk composition of the cows during the trial. However, fat yield was greater ($P < 0.05$) in the OC group than in the CON group, and the fat content tended to be higher in the OC group ($P = 0.087$). No differences were observed in SNF, milk production, or DMI measurements.

Milk FA Composition

The percentages of analyzed FA and their aggregated groups determined in milk collected from CON and OC-treated animals are given in Table 5. The inclusion of OC to the diet of lactating cows significantly increased 18-carbon atom FA content at the expense of a decrease in de novo FA (C <16) concentration. Total SFA as well as individual SFA such as C14:0 and C16:0 were decreased, whereas C18:0 was increased by the addition of OC in the cow diets. Concentrations of the 18-C MUFA, such as oleic acid (C18:1 *cis*-9), as well as the sum of C18:1 *trans*-10 and *trans*-11 were significantly higher in the milk of cows that consumed OC compared with the milk of the CON group. Regarding other MUFA, the content of C14:1 *cis*-9 and C16:1 *cis*-9 acids was reduced and C10:1 *cis*-9 was not affected by the inclusion of OC in the cow diets.

Although no differences in the concentrations of total PUFA were observed between treatments, it is important to mention that milk from the OC group contained a significantly higher concentration of ru-

Table 4. Milk production and chemical composition of milk from cows fed diets with 0 or 10% (DM basis) inclusion of ensiled olive cake for a 4-wk period

Item	Treatment[1]			
	CON	OC	SEM	P-value
DMI, kg/d	25.58	25.56	1.55	NS
Milk yield, kg/d	24.48	25.27	1.29	NS
Fat yield, kg/d	0.80	0.85	0.05	*
Protein yield, kg/d	0.86	0.89	0.04	NS
Milk composition, %				
Fat	3.29	3.40	0.08	†
Protein	3.52	3.53	0.02	NS
SNF	8.62	8.63	0.03	NS

[1]CON = control; OC = part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled olive cake as 10% of DM.

*$P < 0.05$; †$P < 0.1$ (tendency).

| Item | Treatment[1] | | | |
	CON	OC	SEM	P-value
C4:0	3.27	3.23	0.14	NS
C6:0	2.03	2.01	0.05	NS
C8:0	1.27	1.22	0.06	NS
C10:0	2.96	2.65	0.16	NS
C10:1 cis-9	0.33	0.29	0.01	NS
C12:0	3.46	3.00	0.18	NS
C13:0 iso	0.14	0.12	0.009	NS
C14:0	11.50	10.22	0.27	*
C14:1 cis-9	1.44	1.17	0.06	*
C15:0	0.99	0.86	0.04	†
C15:0 iso	0.25	0.27	0.03	NS
C16:0	34.77	30.88	0.50	**
C16:1 cis-9	1.96	1.51	0.06	**
C17:0 ante/iso	0.49	0.39	0.04	†
C17:0	0.49	0.48	0.03	NS
C18:0	8.61	11.76	0.29	***
C18:1 cis-9	18.32	22.17	0.41	**
C18:1 cis-11	0.46	0.61	0.03	*
C18:1 trans-10 + trans-11[2]	0.66	0.92	0.07	*
C18:2n-6	3.55	3.56	0.32	NS
cis-9,trans-11 CLA	0.59	0.65	0.006	*
trans-10,cis-12 CLA	0.26	0.31	0.06	NS
C18:3n-3	0.64	0.47	0.16	NS
C20:3n-6	0.71	0.65	0.08	NS
C20:4n-6	0.92	0.85	0.08	NS
Sum				
SCFA[3]	9.68	9.41	0.21	NS
MCFA[4]	54.81	48.09	0.15	***
LCFA[5]	34.55	41.68	0.12	***
<C16:0	27.81	25.07	0.60	*
>C16:0	34.99	41.97	0.08	***
SFA	70.54	67.07	0.38	**
MUFA	23.12	26.49	0.62	**
PUFA	6.31	6.29	0.42	NS
Atherogenic index[6]	2.93	2.33	0.06	***
Desaturation index[7]	11.13	10.06	0.39	NS

[1]CON = control; OC = part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled olive cake as 10% of DM.

[2]Sum of C18:1 trans-10 and trans-11.

[3]Short-chain fatty acids (C4:0 to C10:1 cis-9).

[4]Medium-chain fatty acids (C12:0 to C16:1 cis-9).

[5]Long-chain fatty acids (C17:0 to C20:4n-6).

[6]Atherogenic index = (C12:0 + 4 × C14:0 + C16:0)/(ΣMUFA + ΣPUFA).

[7]Desaturation index = (C14:1 cis-9/C14:0 + C14:1 cis-9) × 100.

*P < 0.05; **P < 0.01; ***P < 0.001; †P < 0.1 (tendency).

menic acid (**RA**; cis-9,trans-11 CLA) compared with the CON group. However, similar FA content between treatments was observed in trans-10,cis-12 CLA as well as in linoleic (C18:2n-6), α-linolenic (C18:3n-3), and arachidonic (C20:4n-6) acids. The inclusion of OC reduced milk atherogenic index, whereas desaturation index did not differ between treatments. Overall, the differences in the concentrations of individual FA are reflected in differences between the short-chain FA (**SCFA**), medium-chain FA (**MCFA**), and long-chain

FA (**LCFA**), in which OC supplementation reduced MCFA and increased LCFA compared with the CON group, whereas no diet effects were observed on SCFA percentages of milk fat (Table 5).

FA Composition of Halloumi Cheese

The mean percentages of the different FA in Halloumi cheese and the aggregated groups of FA are presented in Table 6. Results showed that Halloumi FA composition was similar to milk FA composition and likewise affected by treatment. Halloumi cheese produced using OC milk contained more 18C FA compared with the cheese of the CON group. At the same time, the percentage of total SFA was decreased (P < 0.01) by the addition of olive by-product because the OC group appeared to have lower values than the CON treatment.

There was no diet effect on C16:1 cis-9, C14:1 cis-9, and MCFA, whereas SCFA were decreased in Halloumi cheese produced by the milk of the OC group. In accordance with milk FA results, MUFA of Halloumi cheese was increased by inclusion of OC. Among individual MUFA, the OC diet increased oleic acid (C18:1 cis-9) and the sum of C18:1 trans-10 and trans-11 acids and decreased the content of C10:1 cis-9 acid.

Regarding PUFA, the average percentage of linoleic acid (C18:2n-6) and cis-9,trans-11 CLA in Halloumi cheese from cows of the OC-supplemented group was increased compared with the CON group. In line with these results, LCFA in Halloumi cheese increased with OC addition compared with the CON group (P < 0.05). In contrast, OC inclusion did not modify the percentages of C18:3n-3, C20:4n-6, C20:3n-6, and total PUFA. Finally, a reduced proportion in atherogenic index was observed in the Halloumi cheese produced with the OC milk (Table 6).

Gene Expression

The mRNA abundance of candidate genes is presented in Figures 1 and 2 for mammary gland and adipose tissues, respectively. In adipose tissue, mRNA expression of *SREBF1* was upregulated in cows fed the OC-supplemented diet. No significant effects were observed in the expression of any of the other genes examined in perirenal fat or in the transcriptional expression of any of the 11 genes analyzed in mammary tissues.

DISCUSSION

The current study was designed to investigate whether partly replacing forages in cow diets with OC silage as 10% of DM based on previous expertise (Hadjipanayiotou, 1999, and Agricultural Research

Institute, Nicosia, Cyprus, personal communication) could improve the milk lipid profile without reducing intake or inducing milk fat depression. Indeed, supplementing the diets of lactating cows with OC did not significantly affect milk fat content similarly to other studies (Hadjipanayiotou, 1999; Meo Zilio et al., 2014; Castellani et al., 2017), whereas an increase in fat yield was demonstrated. In cattle, OC feeding up to 15% on a DM basis did not significantly affect milk fat yield (Hadjipanayiotou, 1999; Meo Zilio et al., 2014). However, in accordance with our study, Castellani et al.

Table 6. Fatty acid composition (expressed as a percentage of total FAME) of Halloumi cheeses made with raw milk from cows fed a diet with 0 or 10% (DM basis) inclusion of ensiled olive cake for a 4-wk period

| | Treatment[1] | | | |
Item	CON	OC	SEM	P-value
C4:0	3.48	2.34	0.18	**
C5:0	0.11	0.05	0.05	NS
C6:0	3.69	2.4	0.08	***
C8:0	3.03	1.32	0.06	*
C9:0	0.14	0.06	0.03	NS
C10:0	4.41	2.27	0.06	*
C10:1 *cis*-9	0.72	0.44	0.01	*
C12:0	3.70	3.71	0.03	NS
C12:1 *cis*-9	0.11	0.12	0.01	NS
C13:0	0.48	0.37	0.06	NS
C14:0	9.31	9.32	0.16	NS
C14:1 *cis*-9	1.58	1.38	0.07	NS
C15:0 iso	0.21	0.52	0.12	NS
C15:0	2.40	1.85	0.02	*
C16:0	25.13	26.49	0.19	NS
C16:1 *cis*-9	2.59	2.37	0.09	NS
C17:0	0.74	0.66	0.12	NS
C17:0 ante/iso	0.66	0.53	0.10	NS
C17:1 *cis*-9	1.28	1.13	0.11	NS
C18:0	7.17	10.18	0.02	***
C18:1 *trans*-10 + *trans*-11[2]	0.46	0.91	0.02	**
C18:1 *cis*-9	18.53	21.53	0.67	***
C18:2n-6	2.65	3.26	0.21	*
cis-9,*trans*-11 CLA	0.36	0.66	0.01	*
C18:3n-3	0.56	0.57	0.02	NS
C20:3n-6	0.17	0.17	0.02	NS
C20:4n-6	0.26	0.31	0.02	NS
Sum				
SCFA[3]	15.81	8.93	0.67	**
MCFA[4]	45.60	45.87	0.47	NS
LCFA[5]	33.15	40.06	1.56	***
SFA	64.90	61.85	0.88	*
MUFA	25.47	27.90	0.55	***
PUFA	4.10	4.88	0.20	NS
Atherogenic index[6]	2.24	2.06	0.04	*

[1]CON = control; OC = part of the forages (alfalfa, barley hay, and barley straw) were replaced with ensiled olive cake as 10% of DM.

[2]Sum of C18:1 *trans*-10 and *trans*-11.

[3]Short-chain fatty acids (C4:0 to C10:1 *cis*-9).

[4]Medium-chain fatty acids (C12:0 to C16:1 *cis*-9).

[5]Long-chain fatty acids (C17:0 to C20:4n-6).

[6]Atherogenic index = (C12:0 + 4 × C14:0 + C16:0)/(ΣMUFA + ΣPUFA).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(2017) found a tendency of increased fat percentage. Although results for fat yield were not reported in that study, higher levels of fat content could have increased fat yield due to the similar milk production reported. In addition, a meta-analysis evaluating the effect of adding free fat or oil supplements in cow diets showed that the effect of dietary lipid profile can be either positive or negative or without any significant difference in milk fat yield, although affecting the FA profile of milk (Dorea and Armentano, 2017).

In the present study, the inclusion of OC in the diets of lactating cows did not affect milk yield, as expected from using isoenergetic and isonitrogenous diets reported previously (Hadjipanayiotou, 1999; Meo Zilio et al., 2014; Castellani et al., 2017), or affect protein content or yield, as has also been found by others (Hadjipanayiotou, 1999; Meo Zilio et al., 2014). Although Castellani et al. (2017) observed that milk protein content was influenced by the integration of dried olive pomace into cow diets, the authors attributed the unexpected results to the difference in the forage:concentrate ratio of the diets.

In our study, addition of ensiled OC in the diets of dairy cows altered milk FA composition and affected individual FA with positive effects for human health. Levels of LCFA and particular FA with 18 carbon atoms such as oleic (C18:1 *cis*-9) and *cis*-9,*trans*-11 CLA were increased, whereas total SFA (particularly C14:0 and C16:0) and, consequently, the atherogenic index were decreased significantly.

The proportion of FA with less than 16 carbons was significantly reduced by replacing part of the forages with ensiled OC. Those results are in line with previous studies that reported reduced de novo FA content, particularly decreased concentration of MCFA, and increased levels of LCFA by addition of a high level of unsaturated 18C FA in cow diets (reviewed in Dorea and Armentano, 2017), as is the case of diets rich in oleic acid in the present and previous studies (He et al., 2012; Castellani et al., 2017; Weld and Armentano, 2018). This could be attributed to a higher uptake of LCFA from the blood or a lower de novo synthesis of FA in the mammary gland (Chilliard et al., 2000; Dorea and Armentano, 2017). The de novo FA synthesis could be inhibited by bioactive FA, including *trans* monoenes and CLA such as *trans*-10,*cis*-12 CLA (Bauman et al., 2006, 2011), which was not, however, significantly different between the 2 examined groups in the present study. The decreased MCFA and C <16 FA, indicative of decreased de novo FA synthesis, could possibly be attributed to isomerization of oleic acid into *trans* monoenes such as C18:1 *trans*-10 and *trans*-11 (Dorea and Armentano, 2017) found significantly elevated in the milk of cows fed ensiled OC in our study; C18:1
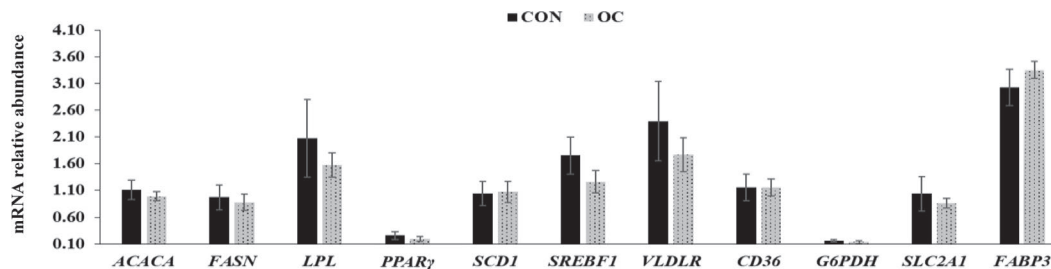
Figure 1. The mRNA relative abundance (mean ± SEM) of lipogenic genes and transcription factors of the mammary tissue in dairy cows fed a control diet (CON; solid black bars) or the control diet supplemented with ensiled olive cake as 10% of DM (OC; dotted gray bars). The mRNA levels are expressed as abundance relative to the geometric mean of *UXT*, *RPS9*, and *RPS15* mRNA.

trans-10 is strongly associated with milk fat depression in cows (Gaynor et al., 1994; Griinari et al., 1998; Shingfield et al., 2009). Although we did not observe a low-fat syndrome, the concentration of FA with less than 16 carbons is a better indicator of inhibition of milk FA secretion compared with milk fat content due to the possible masking effect of the exogenous supply of 18C dietary FA (Dorea and Armentano, 2017). Therefore, both a higher uptake of dietary LCFA and decreased de novo synthesis of MCFA in the mammary glands of cows fed ensiled OC, without affecting milk fat percentage, are possible mechanisms.

Among SFA, a significant increase of stearic acid (C18:0) was observed in milk by OC diet inclusion, which is consistent with previous studies indicating increased levels of C18:0 (He et al., 2012; Castellani et al., 2017) in milk due to high levels of UFA 18C FA in dairy cattle diets. Stearic acid is the final product of biohydrogenation (**BH**) of dietary MUFA and polyunsaturated C18 FA, resulting from microbial BH saturation of double bonds in LCFA (Shingfield et al., 2010; Ferlay et al., 2017), such as oleic acid, which was abundant in the OC diet.

The reduction of SFA in the present study was accompanied by increased total MUFA content in the OC-supplemented group. Similar effects and patterns have been found by researchers who tested diets supplemented with various forms of processed OC in ewes (Chiofalo et al., 2004; Abbeddou et al., 2011a,b, 2015; Vargas-Bello-Pérez et al., 2013; Symeou et al., 2019), goats (Molina-Alcaide et al., 2010), and cows (Castellani et al., 2017). Addition of olive oil in cow diets also led to similar findings (Vargas-Bello-Pérez et al., 2018). It is likely that MUFA of feed, escaping BH, were transferred to milk FA content through mammary uptake from the plasma dietary FA, contributing to the higher MUFA content of milk (Shingfield et al., 2010). In addition, feeding unprotected oils, rich in 18C PUFA, increases both C18:1 and C18:2 *cis* and *trans* isomers arising from ruminal metabolism and from mammary desaturation of C18:0 and C18:1 *trans* (mainly 7 and 11) produced in the rumen (Chilliard et al., 2000). Indeed, milk C18:1 *cis*-9 can be generated either through the action of mammary Δ^9-desaturase with the substrate being stearic acid or by direct transfer from feed (Collomb et al., 2006; Chilliard et al.,
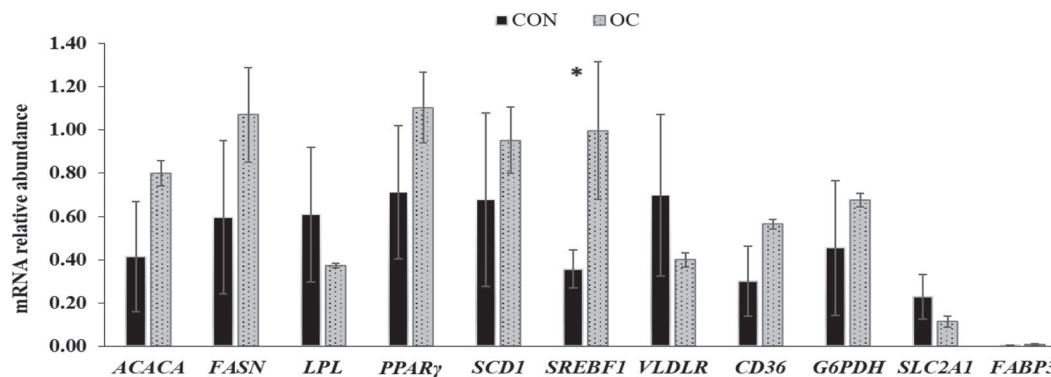

Figure 2. The mRNA relative abundance (mean ± SEM) of lipogenic genes and transcription factors of the adipose tissue in dairy cows fed a control diet (CON; solid black bars) or the control diet supplemented with ensiled olive cake as 10% of DM (OC; dotted gray bars). The mRNA levels are expressed as abundance relative to the geometric mean of *UXT*, *RPS9*, and *RPS15* mRNA. Within each gene, * indicates significant differences ($P < 0.05$) between the experimental treatments.

2007). However, because the Δ^9-desaturation index in the mammary gland did not differ between groups in the current study, it can be assumed that the increase in oleic acid is probably related more to the diet than to Δ^9-desaturase activity.

In the present study, only RA and *trans*-10,*cis*-12 CLA isomers were detectable, from which the former was significantly increased in the milk fat of cows fed the OC diet, whereas the latter did not differ between treatments. Previous studies in cows observed significantly higher contents for both CLA isomers by the addition of dried olive pomace (Castellani et al., 2017) and olive oil (Vargas-Bello-Pérez et al., 2018), whereas in line with our study, He et al. (2012) reported increased levels only for RA by addition of high oleic-rich diets in cows compared with the control group. It is well known that the majority of RA in milk fat is synthesized endogenously in the mammary gland through the action of mammary Δ^9-desaturase with the substrate being vaccenic acid, whereas a small amount of this specific CLA originates from BH of UFA by rumen bacteria (Lock and Bauman, 2004; Collomb et al., 2006; Buccioni et al., 2012). Thus, the elevated proportions of RA observed in the milk of the OC group could be due either to the higher concentrations of vaccenic acid in the mammary gland compared with CON group or to the incomplete BH taking place in the rumen of cows fed the OC diet (Bauman et al., 2006).

Interestingly, the effects of OC treatment in the FA composition of Halloumi cheese were similar to those observed in milk. This suggests that the improvement in nutritional quality achieved in milk because of OC supplementation is thereafter largely maintained in Halloumi cheese. Similarly to the FA profile of milk, reduced levels of SFA and significantly increased levels of LCFA and MUFA were observed in Halloumi cheese when OC was supplemented in cow diet, and, consequently, atherogenic index was diminished. Both milk and Halloumi cheese samples of the OC group had increased content of 18C FA including C18:1 *cis*-9, the sum of C18:1 *trans*-10 and *trans*-11, and RA. Moreover, total PUFA and linolenic acid were not affected in milk or in cheese. However, in contrast to the milk FA content, linoleic acid percentages were elevated in cheese obtained from the OC group. Those results agree with Castellani et al. (2017), who reported that proportions of total MUFA, oleic acid, and RA increased and total SFA decreased in cheese, whereas the percentage of total PUFA was not modified by the inclusion of dried olive pomace in the cow diets. Additionally, studies reported decreased SFA and atherogenic index as well as increased total MUFA, C18:1 *cis*-9, or RA concentrations of cheese fat when 10 and 25% dried OC

(Vargas-Bello-Pérez et al., 2013) or olive oil (Vargas-Bello-Pérez et al., 2018) were included in the diets of ewes and cows, respectively. Furthermore, these studies observed an increase in total PUFA of cheese lipids in diets enriched with oleic acid. It has been reported that heating, the fermentation culture used, and the ripening time required for the type of cheese produced could all potentially modulate cheese FA composition (Collomb et al., 2006; Domagała et al., 2010; Santillo et al., 2016).

This is the first report studying the effects of the inclusion of OC silage on the lipid metabolism of dairy cows. To our knowledge, no other studies have examined potential effects of any form of OC on mammary or adipose gene expression in lactating cows. The current feeding trial used cows in mid lactation. Therefore, tissue samples were obtained after the period of negative energy balance when extensive lipolysis, upregulation of lipolytic genes, and remarked downregulation of lipogenic genes take place (Sumner-Thomson et al., 2011).

During mid lactation and according to our findings, the transcription level of *SREBF1* in adipose tissue was significantly increased in response to the increased level of C18:0 and C18:1 *cis*-9 in the OC diet. However, no effect on the *SREBF1* mRNA abundance of adipose tissue was reported either in nutrigenomic studies conducted in lactating cows after UFA supplementation (Thering et al., 2009; Vahmani et al., 2014) or in a recent in vitro study examining the effect of dose and type of LCFA on adipogenesis of bovine adipocytes (Yanting et al., 2018). In the study of Harvatine et al. (2009), intravenous infusion of *trans*-10,*cis*-12 CLA, a well-established inhibitor of de novo milk FA synthesis, upregulated *SREBF1* in adipose tissue of dairy cows. This particular CLA isomer increases the expression of adipogenic *SREBF1* and lipid synthesis enzymes (reviewed by Bauman et al., 2011), suggesting energy partitioning toward adipose tissue fat stores (Harvatine et al., 2009). In the present study, however, milk fat content was not affected. Supplementation with OC increased the sum of C18:1 *trans*-10 and *trans*-11, the first of which has also been associated with reduced de novo synthesized FA in dairy cows (Dorea and Armentano, 2017). It is likely that increased levels of C18:1 *trans*-10 or other monoenes arising from oleic acid isomerization may have affected the expression of *SREBF1* in adipose tissue of lactating cows, but further research is needed to clarify the particular role of other bioactive CLA and *trans* monoenes on lipid metabolism when oleic acid is fed (Dorea and Armentano, 2017).

Regarding the expression of the remaining genes tested in the adipose tissue, no significant differences were observed between treatments. The transcriptional factor

SREBF1 is a master regulator of lipid synthesis (Osorio et al., 2016); it remains an inactive precursor on the endoplasmic reticulum membrane and is transported to the Golgi for proteolytic cleavage before entering the nucleus, where it activates expression of sterol response element (*SRE*)-containing genes (*ACACA*, *FASN,* and *SCD1*; Bauman et al., 2011; Osorio et al., 2016). Although *SREBF1* was upregulated, we did not observe increased mRNA expression of the genes involved in FA synthesis and desaturation. Similarly, Graugnard et al. (2009) indicated that different levels of starch had no effect on the mRNA expression of *ACACA*, *FASN*, and *SCD1* in adipose tissue of steers despite elevated mRNA expression of *SREBF1*, which could possibly be attributed to posttranscriptional regulation of *SREBF1.* In some studies testing plant oils in bovine (Thering et al., 2009; Vahmani et al., 2014) and caprine (Bernard et al., 2009, 2012) diets, no significant effects on any of the genes expressed in the adipose tissue were reported. The differences of results regarding *SREBF1* expression could be related to the levels of OC inclusion or to other factors that need to be further investigated. For instance, differences in plasma insulin concentrations are known to affect adipose lipogenesis (Vernon, 2005). Overall, the results of this study suggest that the adipose tissue lipogenic pathway does not play an important role in the response of mammary lipid secretion to the OC supplement used.

In the present study, the decreased proportion of the de novo C <16 FA in the cow milk of the OC group was not accompanied by changes in mammary lipogenic gene expression. Feeding cows ensiled OC was not followed by reduction in milk fat percentage or by changes in the expression of any of the genes involved in mammary lipid synthesis despite the decreased proportion of the de novo FA in milk. Additionally, the secretion of LCFA was increased in the OC group, but this was not accompanied by variations in mammary *LPL* mRNA abundance or in mRNA abundance of genes involved in the uptake, transport, and trafficking of FA in the cells (*CD36*, *FABP3*, *VLDLR*, *SLC2A1*). Our results concur with the studies of Bernard et al. (2005, 2009), who observed increased secretion of 18C FA in the milk of goats fed plant oils without effect on the *LPL* mRNA abundance, and with the studies of Bernard et al. (2017) and Fougère and Bernard (2019), who reported no diet effect on the bovine and caprine mammary levels of expression of *LPL*, *SLC2A1*, *CD36*, or *FABP3* by lipid supplementation. It is likely that mammary lipid synthesis, FA uptake, and transport are regulated at the posttranscriptional or posttranslational level for these genes or that short-term regulation of mRNA synthesis by nutrient supply may have taken place (Bernard et al., 2017; Fougère and Bernard, 2019).

CONCLUSIONS

This study examined the inclusion of OC silage (10% of DM) in the diets of lactating Holstein-Friesian cows to determine whether it affects milk and corresponding Halloumi FA composition and to examine the expression of key mammary and adipose lipogenic genes. By supplementing cow diets with OC, we observed in both milk and Halloumi cheese increased levels of individual FA that are related to positive effects on human health (i.e., CLA and oleic acid), whereas SFA were decreased. No variation in the mammary and adipose mRNA abundance due to the diets was observed, apart from *SREBF1*, which was higher in the adipose tissue of the OC group without affecting the expression of the genes *SREBF1* regulates. The results of the present study are useful for exploiting an abundant, cost-effective by-product of the olive oil industry to improve the FA profile of cow milk and derived Halloumi cheese without negative consequences in milk fat content or milk production.

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ORCIDS

M. C. Neofytou ● https://orcid.org/0000-0002-1234-2337

D. Miltiadou ● https://orcid.org/0000-0002-5940-3520

E. Sfakianaki ● https://orcid.org/0000-0001-7636-0182

C. Constantinou ● https://orcid.org/0000-0001-7512-2852

S. Symeou ● https://orcid.org/0000-0002-9844-3517

D. Sparaggis ● https://orcid.org/0000-0002-1461-4033

A. L. Hager-Theodorides ● https://orcid.org/0000-0002-8480-3200

O. Tzamaloukas ● https://orcid.org/0000-0001-7675-6718