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REGULAR ARTICLES

Feeding olive cake silage up to 20% of DM intake in sheep improves lipid quality and health-related indices of milk and ovine halloumi cheese

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Abstract

This study aimed to evaluate the use of a by-product, olive cake silage (OCS), as a forage replacement in sheep diets for the improvement of fatty acid (FA) content of milk and thus, the lipids of the ovine halloumi cheese produced. Sixty second-parity purebred Chios ewes in mid-lactation were assigned to three diet treatments (2 lots of 10 animals per treatment) receiving 0%, 10%, and 20% of OCS on dry matter basis for 3 weeks (treatments S0, S10, and S20, respectively). Halloumi cheese was manufactured from fresh raw milk of ewes fed the three different diets. Inclusion of OCS in the diets increased linearly the concentration in milk of unsaturated FA up to 20%, monounsaturated FA up to 23%, polyunsaturated FA up to 11%, rumenic acid (CLA *cis*-9, *trans*-11) up to 61%, and consequently reduced the atherogenicity and thrombogenicity milk indices by 31% and 27%, for the S10 and S20 treatments, respectively, compared with the control treatment. Moreover, these differences were carried over to the lipid profile of ovine halloumi cheese showing, on average, more than 25% increase of unsaturated, polyunsaturated, and monounsaturated FA, with particularly enhanced oleic and rumenic acid content. These changes resulted in reduced atherogenicity by 29% and 45% and thrombogenicity by 23% and 24% of ovine halloumi cheese made from milk of S10 and S20 diets, respectively. Milk yield, milk fat, or protein content was not affected by S10 or S20 feeding treatments compared to control. Overall, the applied ensiling method of olive cake produces a by-product that can be included as a forage replacement up to 20% of DM intake in Chios sheep without adversely affecting the lactating performance. Furthermore, the present study showed that such substitution improves the lipid quality of milk and related halloumi cheese enriching these ovine dairy products with beneficial to human health fatty acids.

Keywords Ensiled olive cake · Conjugated linoleic acid · Chios sheep · Fatty acids

Introduction

Olive cake (OC) is an abundant by-product of olive oil production that could be included in ruminant diets as an alternative to low-quality forage particularly useful for Mediterranean and tropical areas with shortage of forage (Chiofalo et al. 2004). However, the use of fresh OC in ruminant diets is constrained by seasonal availability of olive oil extraction, which lasts 2 to 3 months, and the rapid deterioration due to lipid oxidation occurring shortly after air exposure. Proposed processing methods such as drying and partly destoned and dried (Abbeddou et al. 2011a, b, 2015), inclusion into multi-nutrient blocks (Molina-Alcaide et al. 2010), or pelleted with other ingredients (Castellani et al. 2017) add extra cost, time, and labor and therefore possibly outweigh its usefulness as an affordable forage substitution alternative. However, a cost-effective method of OC preservation is suggested by researchers in Cyprus (Hadjipanayiotou 1999; Symeou et al. 2019; Neofytou et al. 2020) through silage preparation using fresh OC, shortly after production, without any costs of processing or additives (olive cake silage, OCS), allowing extended storage time and consequently use throughout the year as a forage alternative.

Despite the low nutritive value of this by-product (Hadjipanayiotou 1999; Abbeddou et al. 2015), its use may be promoted to the farmers and subsequently to the halloumi cheese manufacturers due to likely beneficial effects on fat

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quality. Our previous study using this ensiled OC by-product in cow diets at a rate of 10% DM showed improved lipid profile in both bovine milk and related halloumi cheese produced regarding total unsaturated lipids, atherogenicity indices, and particularly beneficial fatty acids (FA) such as conjugated linoleic acid (CLA 18:2 *cis*-9, *trans*-11, rumenic acid (RA)) and oleic acid content (Neofytou et al. 2020). It is not known, however, if higher diet inclusion of OCS on diets may be used on sheep conferring any additional benefits on the quality of ovine milk and ovine halloumi cheese without detriments on milk production and/or intake. Therefore, in the present study, we tested the hypothesis that using ensiled OC feeding in sheep at high rates, up to 20% DM intake as a forage replacement, would positively affect the lipids of both milk and related halloumi cheese, presenting novel data for lipid profile of these ovine dairy products.

Material and methods

Sampling and chemical analysis

Sixty second-parity purebred Chios ewes (bodyweight 52.4 ± 1.74 kg) in mid-lactation were distributed in 6 pens of 10 animals balanced for milk yield and live weight and allocated at random to 3 experimental feeding treatments (2 pens per treatment) for 3 weeks with the following inclusion of OCS in the diets: 0%, (control or S0 treatment), 10% (S10 treatment), and 20% (S20 treatment) on diet DM. These inclusion rates were selected since higher quantities of this oil rich by-product may have detrimental effects on intake, rumen microflora, or milk production (reviewed by Tzamaloukas et al. 2021). For the needs of the current experiment, fresh OC was collected by a three-stage oil mill and ensiled as reported earlier (Neofytou et al. 2020). The experimental diets were iso-nitrogenous and iso-energetic according to ewe maintenance and production requirements and their ingredients and chemical composition can be seen in Table 1. Animals were group fed concentrate and forage twice daily in separate feed troughs, after milking, while the OCS was offered by hand, immediately after morning milking, and were consumed entirely within 15 to 20 min. Animals were housed indoors, feed residues were collected weekly, and a prior 2-week feed adaptation period was applied. All experimental procedures throughout the study were carried out according to national and international guidelines Directive 2010/63/EU and approved by the departmental committee. Milk yield measurements and milk sampling were performed at the end of the third week of OC feeding. For the determination of FA profile in diets, representative bulk feed samples were collected and mixed prior to chemical analysis. For the determination of milk FA composition, milk samples on day 21 of the trial were collected from all ewes (mix morning and evening milking). For the halloumi production, fresh bulk milk was obtained at the end of the trial from each treatment and transformed to halloumi cheese as described earlier (Papademas 2006). Two cheese batches per feeding treatment were produced, and three cheese samples per batch were analyzed for FA content. Lipid isolation from feed, milk, and halloumi samples was performed and FA methyl esters (FAME) were prepared as described previously (Tzamaloukas et al. 2015; Symeou et al. 2020). FA profiles were generated by analyzing the FAME samples on a gas chromatography–mass spectrometer (GCMS-QP2010 Shimadzu, Duisburg, Germany) equipped with an HT280T autosampler (HTA, Brescia, Italy). Subsamples of 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 × 0.25 mm i.d., 0.2 μm film thickness) and the following program was applied: after injection, the column was held for 4 min at 70 °C, increased at 13 °C/min to 175 °C, held at that

Table 1 Ingredients and chemical composition of the experimental diets S0, S10, and S20 with olive cake silage (OCS) inclusion at rates of 0, 10, and 20% of DM, respectively. Chemical composition of OCS is also shown

Item	Experimental diets			OCS
	S0	S10	S20	
Ingredients, % of DM				
Barley hay	17.71	17.54	17.37	
Lucerne hay	8.75	8.66	8.58	
Barley straw	18.27	9.04	0.00	
Olive cake silage	0.00	10.02	19.85	
Barley grain	15.26	15.11	14.96	
Corn	11.06	10.95	10.84	
Wheat bran	4.42	4.38	4.34	
Sugar beet pulp	6.63	6.57	6.50	
Sunflower meal (35% CP)	5.53	5.47	5.42	
Distillers dark grain (wheat)	4.42	4.38	4.34	
Soybean meal (47% CP)	5.97	5.91	5.85	
Molasses	0.55	0.55	0.54	
Limestone	0.99	0.99	0.98	
Monocalcium phosphate	0.06	0.05	0.05	
Salt	0.28	0.27	0.27	
Vitamins and trace mineral mix	0.11	0.11	0.11	
Composition, % of DM				
CP	15.01	15.08	15.14	5.82
Crude fiber	19.86	19.85	19.84	56.88
Ash	6.71	6.40	6.10	2.51
Ether extract	2.36	3.19	4.01	10.84
NDF	40.70	40.40	40.20	78.45
ADF	25.90	25.01	24.30	66.21
Energy, NEL Mj/kg DM	10.44	10.37	10.30	

NEL, net energy lactation calculated according to NRC (2001)

temperature for a further 27 min and then raised to 215 °C at 4 °C/min, at which it was held for further 36 min. Helium was the carrier gas at 1 mL/min, with both injector and interface temperatures at 225 °C. FA profiles were analyzed using Shimadzu GCMS Post-run Solution software with the National Institute of Standards and Technology 08 and 21 mass spectral libraries and cross referencing with chromatograms and spectrograms reported in the literature.

Statistical methods

The results were analyzed by the statistical program JMP PRO Ver 14.2 (SAS Institute Inc., NC, USA) after verification of the assumptions, including normality. The analysis for the dependent variables of milk yield, milk composition, and FA percentages in total fat of feed, milk, and halloumi cheese with independent variables were evaluated with the mathematical model:

$$Y_{ijl} = \mu + \alpha_i + d_{ij} + e_{ijl}$$

where Y_{ijl} is the value of the response-dependent variable for the ith feeding treatment, in the jth lot (or cheese batch) from the lth ewe (or cheese sample); μ is the overall mean response; α_i is the fixed main effect of treatment (level $i = 0, 1, 2$); d_{ij} is the random effect of treatment nested in lot (lot level $j = 1, 2$) distributed $N(0,\sigma_d^2)$; and ε_{ijl} is the random residual error effect of lth ewe (level $l = 1, ..., 10$) on the ith treatment in the jth lot (or batch for halloumi) distributed $N(0,\sigma_e^2)$. The significance of the effects was evaluated as follows: the treatment effect was decomposed into two orthogonal polynomial contrasts, linear (L) and quadratic (Q), and are numerically presented in tables when a significance (P-values less than 0.05) or a tendency for significance (P-values less than 0.1) was found.

Results

The chemical content and the FA composition of the experimental diets on offer are presented in Tables 1 and 2, respectively. Regarding the feed FA composition, the most abundant individual FA in OCS was oleic acid (C18:1 *cis*-9; almost 2/3 of total fat), followed to a lesser content by palmitic (C16:0) and linoleic (C18:2 *cis*-9, *cis*-12) acids. As a consequence, the inclusion of OCS resulted in a remarkable change in the composition of lipids of the three diets regarding almost all individual FA identified. During the experiment, the feed intake, milk yield, or milk composition regarding fat, protein, and total solids were not affected by the feeding treatment (data shown in Table 3).

Table 4 shows the FA composition of milk samples collected at the end of the 3-week feeding period. OCS feeding resulted in marked modifications in milk FA compared with the control. Diet inclusion of OCS resulted to a linear increase of MUFA class in milk to the detriment of most saturated fats in milk, including odd and branch-chain FA, with profound reduction in the major SFA such as C8:0, C10:0, C12:0, C14:0, and C16:0, except C18:0, which was increased with the OCS inclusion in the diets. Oleic acid, other C18:1 *cis*-isomers, and C18:1 *trans*-10 were particularly enhanced ($P < 0.001$), whereas C18:1 *trans*-11 (vaccenic acid, VA) was not affected by OCS feeding. Regarding 18-carbon dienes in milk, percentages of linoleic acid and all non-conjugated C18:2 isomers increased with the OCS participation in the diets. Major CLA, C18:2 *cis*-9, *trans*-11 (RA) was linearly increased by 42% and 61% when the diets included 10 and 20% of OCS, respectively, but no effect was observed for the other conjugated FA identified, namely C18:2 *trans*-10, *cis*-12. Other

Table 2 Fatty acid composition (g/100 g of total fatty acid methyl esters) of olive cake silage (OCS) and the experimental diets S0, S10, and S20 with different inclusion of OCS (0, 10, and 20% on DM, respectively)

Item	OCS	Experimental diets			SEM	Effect of treatment[1]	
		S0	S10	S20		*L*	*Q*
C14:0	0.03	1.11	0.60	0.30	0.03	0.005	0.227
C16:0	13.57	19.12	16.79	15.64	0.17	<0.001	0.124
C18:0	3.83	4.15	3.75	3.55	0.02	<0.001	0.205
C18:1 *cis*-9	64.49	24.02	39.23	46.75	0.66	<0.001	0.007
C18:2 n-6	12.95	39.15	30.13	25.68	0.35	<0.001	0.007
C18:3 n-3	1.37	8.21	5.62	4.34	0.05	<0.001	<0.001
C20:0	1.10	1.29	1.06	0.94	0.18	NS	

[1] *L*, probability of linear effect; *Q*, probability of quadratic effect; *NS*, non-significant effect, nor tendency (P value > 0.1)

Table 3 Feed intake, milk production, and chemical composition of milk from ewes fed diets with olive cake silage inclusion at rates of 0 (control, S0), 10 (S10), and 20% of DM

Item	Experimental diets			SEM	Effect of treatment
	S0	S10	S20		
Intake, DM kg/d	2.52	2.44	2.56	0.70	NS
Yield, g/d					
Milk	1933	1960	2037	49.30	NS
Fat	86	86	87	5.38	NS
Protein	84	87	88	4.81	NS
Total solids	311	318	322	17.66	NS
Composition, %					
Fat	4.46	4.37	4.30	0.14	NS
Protein	4.36	4.43	4.35	0.03	NS
Total solids	16.08	16.17	15.90	0.16	NS

NS, non-significant effect nor tendency (P value > 0.1)

Table 4 Fatty acid profile (g/100 g of total fatty acid methyl esters) of milk fat from ewes fed diets with olive cake silage (OCS) inclusion at rates of 0% (control, S0), 10% (S10), and 20% (S20) of DM

Item	Experimental diets			SEM	Effect of treatment[1]	
	S0	S10	S20		*L*	*Q*
Saturated fatty acids						
C4:0	1.71	2.10	2.07	0.14	0.002	0.023
C6:0	2.08	2.03	1.94	0.09	0.059	0.821
C8:0	2.34	2.07	2.00	0.12	<0.001	0.174
C10:0	5.53	4.53	4.43	0.29	<0.001	0.029
C12:0	4.15	3.31	3.21	0.17	<0.001	0.003
C13:0	0.19	0.16	0.14	0.02	<0.001	0.209
iso-C14:0	0.28	0.24	0.20	0.01	<0.001	0.836
C14:0	9.25	7.62	7.44	0.27	<0.001	<0.001
iso-C15:0	0.61	0.54	0.45	0.03	<0.001	0.606
anteiso-C15:0	0.95	0.87	0.76	0.04	<0.001	0.625
C15:0	1.69	1.45	1.31	0.05	<0.001	0.117
iso-C16:0	0.73	0.67	0.58	0.05	<0.001	0.171
C16:0	20.37	18.25	17.82	0.69	<0.001	0.029
C17:0	1.11	0.99	0.92	0.04	<0.001	0.439
C18:0	12.78	14.22	14.32	0.49	<0.001	0.040
C20:0	0.79	0.74	0.71	0.04	NS[9]	
C22:0	0.41	0.34	0.34	0.05	NS	
C24:0	0.11	0.10	0.11	0.01	NS	
Monounsaturated fatty acids						
C10:1 *cis*-9	0.38	0.33	0.33	0.03	0.039	0.326
C14:1 *cis*-9	0.28	0.25	0.24	0.02	0.030	0.652
C16:1 *cis*-9	1.18	1.14	1.05	0.08	0.033	0.684
C17:1 *cis*-9	0.37	0.38	0.35	0.03	NS	
C18:1 *trans*-10	0.38	0.78	0.83	0.15	<0.001	0.102
C18:1 *trans*-11	1.34	1.59	1.53	0.19	NS	
C18:1 *cis*-9	17.72	20.79	20.97	0.57	<0.001	<0.001
C18:1 *cis* other	1.69	2.14	2.51	0.10	<0.001	0.526
Polyunsaturated fatty acids						
C18:2 *trans*-8, *cis*-12/*cis*-9, *trans*-13	0.19	0.51	0.63	0.04	<0.001	<0.001
C18:2 *trans*-8, *cis*-13	0.12	0.20	0.25	0.03	<0.001	0.283
C18:2 *trans*-9, *cis*-12	0.06	0.09	0.13	0.02	<0.001	0.663
C18:2 *cis*-9, *cis*-12 (n-6)	3.18	3.59	3.80	0.24	<0.006	0.777
C18:3 (n-3)	0.47	0.44	0.44	0.07	NS	
CLA *cis*-9, *trans*-11	0.75	1.07	1.21	0.08	<0.001	0.065
CLA *trans*-10, *cis*-12	0.30	0.16	0.16	0.09	0.078	0.244
C20:3 (n-6)	0.22	0.14	0.15	0.08	NS	
C20:4 (n-6)	0.49	0.40	0.35	0.05	<0.001	0.598
Fatty acid groups						
SFA[2]	67.33	62.68	59.86	1.26	<0.001	0.170
<C16:0	29.78	25.79	24.71	0.93	<0.001	0.022
>C16:0	45.33	51.14	52.53	1.40	<0.001	0.007
UFA[3]	32.24	36.58	38.99	1.26	<0.001	0.150
MUFA[4]	25.44	29.44	31.41	0.93	<0.001	0.055
PUFA[5]	6.80	7.14	7.58	0.40	0.007	0.845
AI[6]	2.07	1.54	1.41	0.05	<0.001	0.002
TI[7]	2.74	2.13	2.00	0.12	<0.001	0.002
DI[8]	2.92	3.20	3.13	0.14	NS	

[1] *L*, probability of linear effect; *Q*, probability of quadratic effect

[2] Saturated fatty acids

[3] Unsaturated fatty acids

[4] Monounsaturated fatty acids

[5] Polyunsaturated fatty acids

[6] Atherogenicity index [C12:0 + (4 × C14:0) + C16:0] / [MUFA + PUFA(n-3:n-6)]

[7] Thrombogenicity index (C14:0 + C16:0 + C18:0 / (0.5 × MUFA) + [0.5 × PUFA(n-6)] + [3 × PUFA(n-3)] + (n-3/n-6)

[8] Desaturation index (C14:1 × 100) / (C14:1 + C14:0)

[9] *NS*, non-significant effect nor tendency (*P* value > 0.1)

PUFA were not affected or even decreased with OCS diets, such as the arachidonic acid (C20:4 n-6) content.

The observed milk lipid differences were carried over to the lipid profile of ovine halloumi cheese, which can be seen in Table 5. Diet OCS inclusion increased MUFA and PUFA with concomitant reduction of total SFA content. All individual SFA determined in cheese were linearly diminished with the addition of OCS in the ewe diets, except butyric acid (C4:0) which was not affected and stearic acid (C18:0) which was increased in the halloumi cheese of S20 treatment. In contrast to the SFA, total percentages of MUFA and UFA were both increased more than 25% in S10 and S20 diets compared to control. With regards to MUFA, its increase is attributed to the percentages of 18-carbon monoene isomers (oleic, vaccenic, and C18:1 *trans*-10 acids), since other MUFA (C10:1 *cis*-9, C14:1 *cis*-9, C16:1 *cis*-9, C17:1 *cis*-9) were reduced with the inclusion of OCS. Concerning individual PUFA, OCS inclusion resulted in an increase in all 18-carbon dienes, including linoleic and RA, with the latter particularly enhanced, demonstrating a 2-fold increment in S20 treatment. Other UFA in halloumi cheese such as linolenic and arachidonic acid were reduced in the OCS treatment.

Regarding the health-related indices in milk, the inclusion of OCS reduced the atherogenicity index by 25% and 31% and the thrombogenicity index by 22% and 27%, in S10 and S20 treatments, respectively, compared to control. Similarly, in the corresponding halloumi cheese, atherogenicity and thrombogenicity indices were reduced by 29% and 23% in S10 and by 45% and 24% in S20 treatment, respectively, compared to control.

Discussion

The current study investigated the effects of OCS feeding in the dairy Chios sheep, focusing on the FA profile of milk and corresponding ovine halloumi cheese. Overall, the inclusion of OCS in the sheep diets resulted in reduction of the total SFA percentages, and particularly the group of SFA with less than 18-carbons, accompanied by a significant increase of total MUFA, oleic acid, and total UFA in both milk and halloumi cheese. These results agree with the findings of other researchers on milk, cheese, and yoghurt by the utilization of fresh or processed forms of OC in ewes (Abbeddou et al. 2011a, b), goats (Molina-Alcaide et al. 2010), and cows (Castellani et al. 2017; Neofytou et al. 2020) and will be discussed in detail, focusing to those lipids with particular interest to human health.

Regarding the content of stearic and oleic acids, both were higher in milk and halloumi cheese for the OCS groups compared to the control. Previous studies with the use of different OC processed forms (Chiofalo et al. 2004; Abbeddou et al. 2011a, b, 2015) also reported higher contents of stearic and oleic acids in milk and cheese. Milk stearic acid can be derived from both the feed, directly transferred into milk, and the rumen, as the final hydrogenation product of the C18 MUFA and PUFA (Doreau et al. 2016). Since OCS feeding treatments in the present study had lower stearic content and significantly higher oleic content compared to the control diet, it is suggested that the mechanism of bio-hydrogenation has been involved. In the case of oleic acid, it has been reported that this FA may well originate from the mammary uptake of dietary oleic acid but also derived by the desaturation of stearic acid from Δ-9 desaturase in the rumen or mammary gland (Doreau et al. 2016). In our study, the desaturation index was not affected by any inclusion rates of OCS in the diet suggesting that the transfer from the rich in oleic acid diets may have played the major role.

Regarding RA, the inclusion at 10 and 20% OCS affected linearly and positively the levels of RA in milk and, consequently, the RA content in halloumi cheese. These findings agree with previous works in sheep milk (Symeou et al. 2019), in bovine milk, and halloumi cheese (Neofytou et al. 2020) and other studies with inclusion of dried OC in bovine milk and related cheese (Castellani et al. 2017) and caprine milk (Molina-Alcaide et al. 2010). However, studies exist reporting no changes in the RA content neither for ovine milk (Abbeddou et al. 2011b) nor ovine yoghurt or cheese (Abbeddou et al. 2011a) following supplementation with dried OC. The different results on RA content in ruminant milk after OC feeding may be attributed to the different process of OC (fresh, dried, ensiled), other feed ingredients of the diet, and/or supplementation rates of OC by-product, which all could result in altering also rumen microflora (reviewed by Tzamaloukas et al. 2021). In our study, the inclusion of 10 or 20% of OCS for a 3-week period resulted in significant increase in milk RA and this was transferred to the ovine halloumi cheese produced for both treatments.

Regarding other polyunsaturated FA, the OCS inclusion in ewe diets has positively influenced the linoleic acid in milk and halloumi cheese in the present study. Increased contents of linoleic acid were also reported in ovine milk (Symeou et al. 2019) when ewes were fed OCS, while reduction was reported with dried OC (Abbeddou et al. 2011b) or with no effects when partly destoned fresh OC was included in the ewe diets (Chiofalo et al. 2004). As far as linolenic acid content in milk is concerned, there was no significant response to the OCS in the present study. However, the linolenic acid content in halloumi cheese, made of the S10 and S20 milk groups, was lower compared to the control and it was the only FA that did not follow the milk FA pattern, with unclear etiology. Nevertheless, the results from other researchers are contradictory since supplementing diets with dried OC or partly destoned fresh OC reported no influence or reduction of the linolenic acid content in milk and cheese fat (Abbeddou et al. 2011b; Chiofalo et al. 2004; Tzamaloukas et al. 2021).

Table 5 Fatty acid profile (g/100 g of total fatty acid methyl esters) of halloumi cheese made with raw milk from ewes fed diets with olive cake silage (OCS) inclusion at rates of 0% (control, S0), 10% (S10), and 20% (S20) of DM

	Experimental diets			SEM	Effect of treatment[1]	
	S0	S10	S20		*L*	*Q*
Saturated fatty acids						
C4:0	1.56	1.60	1.74	0.09	0.061	0.471
C6:0	1.78	1.64	1.55	0.03	<0.001	0.379
C8:0	2.02	1.75	1.55	0.04	<0.001	0.291
C10:0	5.60	4.59	3.71	0.20	<0.001	0.630
C12:0	4.59	3.53	2.90	0.10	<0.001	0.025
C13:0	0.13	0.09	0.07	0.00	<0.001	0.017
iso-C14:0	0.21	0.14	0.11	0.01	<0.001	0.001
C14:0	10.80	9.17	7.96	0.21	<0.001	0.209
iso-C15:0	0.49	0.30	0.25	0.01	<0.001	<0.001
anteiso-C15:0	0.90	0.59	0.50	0.02	<0.001	<0.001
C15:0	1.42	1.06	0.88	0.03	<0.001	0.006
iso-C16:0	0.46	0.32	0.30	0.02	<0.001	0.004
C16:0	24.14	23.32	20.79	0.58	<0.001	0.082
C17:0	0.73	0.52	0.51	0.04	<0.001	0.015
C18:0	13.59	13.16	17.30	0.24	<0.001	<0.001
C20:0	0.45	0.37	0.49	0.02	NS[8]	
C22:0	0.16	0.12	0.17	0.01	NS	
C24:0	0.04	0.09	0.05	0.01	NS	
Monounsaturated fatty acids						
C10:1	0.26	0.19	0.17	0.01	<0.001	0.006
C14:1 *cis*-9	0.22	0.15	0.13	0.01	<0.001	0.005
C16:1 *cis*-9	1.02	0.83	0.67	0.02	<0.001	0.498
C16:1 *cis*-9	0.17	0.10	0.07	0.01	<0.001	0.002
C17:1 *cis*-9	0.25	0.17	0.14	0.01	<0.001	0.002
C18:1 *cis*-9	21.39	27.83	29.83	0.48	<0.001	<0.001
C18:1 *trans*-10	0.32	0.64	0.63	0.10	0.011	0.067
C18:1 *trans*-11	0.30	0.56	0.63	0.05	<0.001	0.035
Polyunsaturated fatty acids						
C18:2 *trans*-9, *trans*-12	0.09	0.23	0.25	0.06	0.018	0.195
C18:2 *cis*-9, *cis*-12	2.80	3.48	3.23	0.15	0.014	0.005
C18:2 *cis*-9, *trans*-11 CLA	0.41	0.74	0.86	0.03	<0.001	0.004
C18:3 (n-3)	0.28	0.24	0.20	0.02	0.001	0.899
C20:4 (n-6)	0.22	0.19	0.16	0.01	<0.001	0.345
Fatty acid groups						
SFA[2]	70.87	63.52	61.95	0.96	<0.001	0.005
<C16:0	30.47	25.02	21.69	0.51	<0.001	0.262
>C16:0	43.3	49.9	55.9	0.97	<0.001	0.636
UFA[3]	29.13	36.48	38.05	0.96	<0.001	0.005
MUFA[4]	25.17	31.54	33.08	0.70	<0.001	0.003
PUFA[5]	3.96	4.94	4.71	0.27	0.015	0.020
AI[6]	2.51	1.78	1.43	0.08	<0.001	0.019
TI[7]	3.21	2.47	2.42	0.11	<0.001	0.004

[1] *L*, probability of linear effect; *Q*, probability of quadratic effect

[2] Saturated fatty acids

[3] Unsaturated fatty acids

[4] Monounsaturated fatty acids

[5] Polyunsaturated fatty acids

[6] Atherogenicity index [C12:0 + (4 × C14:0) + C16:0] / [MUFA + PUFA(n-3:n-6)]

[7] Thrombogenicity index (C14:0 + C16:0 + C18:0 / (0.5 × MUFA) + [0.5 × PUFA(n-6)] + [3 × PUFA(n-3)] + (n-3/n-6)

[8] *NS*, non-significant effect nor tendency (*P* value > 0.1)

Overall, the present study confirms our hypothesis that ensiled olive cake by-product can be used for improvement of both milk and halloumi cheese lipids. This OC silage can provide an applicable forage replacement in sheep diets at rates up to 20% of DM without adversely affecting intake, milk yield, or milk contents, as observed previously with other forms of processed OC (reviewed by Tzamaloukas et al. 2021). Moreover, this substitution affects positively the lipid quality of ovine milk and halloumi cheese produced with decreased saturation, atherogenicity, and thrombogenicity and

concomitant increased MUFA and CLA content. The improved health indices support the use of this by-product in sheep diets as a forage substitution in Mediterranean and tropical areas with shortages in available roughages.

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Availability of data and material The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable to this work.

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Declarations

Ethics approval All experimental procedures were carried out according to the international guidelines (Directive, 2010/63/EU) and approved by the corresponding departmental committee of the Cyprus University of Technology.

Consent to participate All authors have significantly contributed and gave their informed consent prior to their inclusion in the study.

Consent for publication All authors are in agreement with the content of the manuscript to be published.

Conflict of interest The authors declare no competing interests.

References

Abbeddou, S., Rischkowsky, B., Hilali, M.E.D., Hess, H.D. and Kreuzer, M., 2011a. Influence of feeding Mediterranean food industry by-products and forages to Awassi sheep on physicochemical properties of milk, yoghurt and cheese. Journal of Dairy Research, 78, 426–435.

Abbeddou, S., Rischkowsky, B., Richter, E.K., Hess, H.D. and Kreuzer, M., 2011b. Modification of milk fatty acid composition by feeding forages and agro-industrial byproducts from dry areas to Awassi sheep. Journl of Dairy Science, 94, 4657–4668.

Abbeddou, S., Rischkowsky, B., Hilali, M.E.D., Haylani, M., Hess, H.D. and Kreuzer, M., 2015. Supplementing diets of Awassi ewes with olive cake and tomato pomace: on-farm recovery of effects on yield, composition and fatty acid profile of the milk. Tropical Animal Health and Production, 47, 145–152. https://doi.org/10.1007/s11250-014-0699-x

Castellani, F., Vitali, A., Bernardi, N., Marone, E., Palazzo, F., Grotta, L. and Martino, G., 2017. Dietary supplementation with dried olive pomace in dairy cows modifies the composition of fatty acids and the aromatic profile in milk and related cheese. Journal of Dairy Science, 100, 8658–8669.

Chiofalo, B., Liotta, L., Zumbo, A. and Chiofalo, V., 2004. Administration of olive cake for ewe feeding: Effect on milk yield and composition. Small Ruminant Research, 55, 169–176.

Directive, 2010/63/EU Of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. L 276/ 33, 20.10.2010., n.d.

Doreau, M., Meynadier, A., Fievez, V. and Ferlay, A., 2016. Ruminal metabolism of fatty acids: modulation of polyunsaturated, conjugated, and trans fatty acids in meat and milk. In: Handbook of Lipids in Human Function, Chapter 19, 521-542.

Hadjipanayiotou, M. 1999. Feeding ensiled crude olive cake to lactating Chios ewes, Damascus goats and Friesian cows. Livest. Prod. Sci. 59:61–66. https://doi.org/10.1016/S0301-6226(99)00005-6.

Molina-Alcaide, E., Morales-García, E.Y., Martín-García, A.I., Ben Salem, H., Nefzaoui, A. and Sanz-Sampelayo, M.R., 2010. Effects of partial replacement of concentrate with feed blocks on nutrient utilization, microbial N flow, and milk yield and composition in goats. Journal of Dairy Science, 93, 2076–2087.

Neofytou, M.C., Miltiadou, D., Sfakianaki, E., Constantinou, C., Symeou, S., Sparaggis, D., Hager-Theodorides, A.L. and Tzamaloukas, O., 2020. 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. Journal of Dairy Science, 103, 8998–9011. https://doi.org/10.3168/jds.2020-18235

Papademas, P., 2006. Halloumi cheese. In: Brined Cheeses, ed. A.Y. Tamime, Chapter 4, Blackwell Publishing Ltd, Oxford, 117-138.

Symeou, S., Tsiafoulis, C.G., Gerothanassis, I.P., Miltiadou, D. and Tzamaloukas, O., 2019. Nuclear magnetic resonance screening of changes in fatty acid and cholesterol content of ovine milk induced by ensiled olive cake inclusion in Chios sheep diets. Small Ruminant Research, 177, 111–116.

Symeou, S., Tzamaloukas, O., Banos, G. and Miltiadou, D., 2020. ACAA2 and FASN polymorphisms affect the fatty acid profile of Chios sheep milk. Journal of Dairy Research, Article in press

Tzamaloukas, O., Orford, M., Miltiadou, D. and Papachristoforou, C., 2015. Partial suckling of lambs reduced the linoleic and conjugated linoleic acid contents of marketable milk in Chios ewes. Journal of Dairy Science, 98, 1739–1749.

Tzamaloukas, O., Neofytou, M.C. and Simitzis, P.E. 2021. Application of Olive By-Products in Livestock with Emphasis on Small Ruminants: Implications on Rumen Function, Growth Performance, Milk and Meat Quality. Animals, 11, 531. https://doi.org/10.3390/ani11020531