Tropical Animal Health and Production (2021) 53:257
https://doi.org/10.1007/s11250-021-02706-2

REGULAR ARTICLES

Short-term forage substitution with ensiled olive cake increases beneficial milk fatty acids in lactating cows

Marina C. Neofytou[1] · Despoina Miltiadou[1] · Simoni Symeou[1] · Dionysis Sparaggis[2] · Ouranios Tzamaloukas[1]

Received: 24 September 2020 / Accepted: 3 April 2021

Abstract

This study aimed to evaluate the effect of short-term forage substitution with ensiled olive cake (OC), on yield, composition and fatty acid (FA) profile of cows' milk. Mid-lactating Holstein-Friesian cows were randomly assigned for 21 days to two isoenergetic and isoproteic feeding treatments (12 animals per treatment), containing 0 and 10% DM of ensiled OC (C and OC groups, respectively). Milk yield was recorded daily, and milk samples were collected at 14 and 21 days of the trial for analyzing the fat, protein, and FA profile of milk. No significant differences were observed in milk yield, protein, and fat nor in protein and fat percentage of milk between groups. However, dietary supplementation with ensiled OC modified the FA profile of cow milk. Feeding cows with ensiled OC resulted in a significant decline of medium-chain FA, while long-chain and mono-unsaturated FA were risen in milk ($P < 0.05$). Among individual saturated FA, palmitic was particularly reduced, while among individual mono-unsaturated FA, increments of C18:1 *cis*-9 were demonstrated with the OC treatment ($P < 0.05$). Although total poly-unsaturated FA were decreased, the concentration of CLA *cis*-9, *trans*-11 tended to be elevated with OC feeding ($P = 0.06$). Overall, short-term forage substitution with ensiled OC improved, beneficially for human health, the lipid profile of milk without adversely affecting milk yield or milk composition of lactating cows.

Keywords Ensiled olive cake · Milk fatty acid · Milk yield · Cow

Introduction

Inclusion of different products rich in unsaturated fatty acids (UFA) in the diets of lactating ruminants alters milk fatty acid (FA) composition beneficially for human health (Ferlay et al. 2017). Crude olive cake (OC), which is the most abundant by-product of olive oil production, represents an alternative forage substitution and could be useful for tropical and Mediterranean areas with shortage of roughages (Costa et al. 2019). Nevertheless, the inclusion of OC in ruminants' feed is now rare because of its low nutritive value, rapid spoilage, due to rancidity, and seasonal availability (Hadjipanayiotou 1999; Tzamaloukas et al. 2021), while the process of drying or destoning adds extra costs to this by-product. Considering the disadvantages of using fresh or dried OC, the ensiling method applied in Cyprus is a cost-effective alternative to overcome those difficulties, allowing the use of OC in ruminants throughout the year. Moreover, OC may improve the lipids of milk due to its richness in oleic acid. Studies performed in dairy sheep demonstrated a decrease in saturated fatty acids (SFA) and an increase in monounsaturated FA (MUFA) and, in some cases, increased specific beneficial UFA, such as conjugated linoleic acid (CLA), content of milk by the inclusion of dried (Abbeddou et al. 2011a, b, 2015), partly destoned fresh (Chiofalo et al. 2004) or ensiled OC (Symeou et al. 2019, 2021). Recent studies in dairy cows reported similar results. Castellani et al. (2017) showed increased MUFA and CLA and decreased SFA content in milk of cows fed dried olive pomace for a long feeding period of 64 days, while our previous work demonstrated that ensiled OC can improve the FA content of milk and related Halloumi cheese without adversely affecting the expression of genes involved in lipid metabolism of mammary and adipose tissues in cows (Neofytou et al. 2020). To date, no study has examined the effect of OC diet inclusion as a forage substitution for a short-term feeding period in lactating cows. This

✉ Ouranios Tzamaloukas
ouranios.tzamaloukas@cut.ac.cy

[1] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, P.O Box 50329, Limassol, Cyprus

[2] Agricultural Research Institute, P.O. Box 22016, Nicosia, Cyprus

Published online: 12 April 2021

information would be useful in farming practices of specific areas during periods of forage shortages or to produce a special dairy product. Therefore, the objective of this study was to assess the short-term inclusion of 10% (DM) of ensiled OC into the diets of lactating dairy cows, on milk production, protein and fat content as well as on milk FA composition.

Materials and methods

Animals and diets

The experiment was performed in the Agricultural Research Institute of Cyprus according to national and international guidelines (Directive, 2010/63/EU 2010). Twenty-four, mid-lactating Holstein–Friesian cows (average BW ± SD: 611 ± 45) were distributed in 6 pens of 4 animals each and allocated randomly to 2 experimental feeding treatments (3 pens per treatment). The control group (C) 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 47.6%), obtaining a concentrate to forage ratio of 64:36 in both treatments. The two feeding treatments were iso-energetic and iso-nitrogenous and were offered for a 10-day adaptation period followed by 21 days recording and sampling period (Table 1). The OC prepared according to the method described by Symeou et al. (2019). Forages (barley hay, barley straw, and alfalfa hay) were prepared separately for the two treatments (control and OC) with mix wagon and offered manually per pen (group fed) two times per day after morning and evening milking. The OC prepared according to the method described by Symeou et al. (2019) and offered manually directly after morning milking, and cows consumed it entirely within about 15 min. Concentrate mix was offered individually to each cow, 4 times per day, via automatic feeders (Westfalia, Albersdorf, Germany) placed in each pen.

Measurements, sampling, and analysis

Dry matter intake (DMI) was monitored daily, for forages by manually weighing the offered diets and collected leftovers and for concentrate mixture with automatic measurements collected by the electronic system (Crystal Herd Management Software, Fullwood LTD) for each cow. The measurements collected at 14 and 21 days of the experiment were used for statistical analysis of the DMI. Representative feed samples of ensiled OC, of forage mixture including alfalfa hay, barley hay, barley straw, and of concentrate mix on offer were collected at the beginning and at the end of the trial, mixed per treatment, and samples were taken for analysis. The chemical composition of the 2 treatments is presented in Table 1. Dry matter, ash, crude fat, and crude protein were determined as described by AOAC (2005). Crude fiber, ADF, ADL, and amylase NDF (aNDF) were measured according to the method of van-Soest et al. (1991).

Cows were machine milked twice daily and milk production was recorded. Raw milk samples for the determination of the milk composition and lipid profile were collected at 14 and 21 days from each cow during the two consecutive milkings (morning and evening). Measurements for total fat and protein of milk were determined through methods described previously (Tzamaloukas et al. 2015). Lipids from milk and feeds were extracted and methylated as described by Symeou et al. (2019, 2020), respectively. Analyses of FA methyl esters (FAME) of experimental diets and milk samples were performed by using a GCMS-QP2010 Plus Gas Chromatography-Mass Spectrometer (Shimadzu, Duisburg, Germany) equipped with a 100 m × 0.25 mm × 0.2 μm column (Agilent CP-Sil 88 fused silica capillary column) with a 1:20 split ratio. The column was

Table 1 Ingredients, chemical composition, and fatty acid profile of dietary Treatments and the olive cake silage used

Item	Treatment		
	C	OC	Ensiled OC
Ingredient composition (%)			
Ensiled OC	-	10	
Alfalfa	7	7	
Barley hay	18	13	
Barley straw	11	6	
Concentrate mix[a]	64	64	
Chemical composition (% DM)			
Dry matter, %	92.9	83.83	47.58
Crude protein, % DM	17.24	17.22	5.45
Crude fat, % DM	1.75	2.29	6.93
Crude fiber, % DM	17.35	18.72	49.00
Ash, % DM	6.56	6.02	2.51
aNDF, % DM	35.74	36.44	71.46
ADL, % DM	3.5	5.62	28.25
ADF, % DM	21.12	22.4	54.62
Metabolized energy (MJ/kg)[b]	9.2	9.41	-
Fatty acid profile, % of total fatty acid			
C16:0	26.48	23.58	13.45
C16:1 *cis*-9	0.32	0.61	1.48
C18:0	1.91	2.55	4.15
C18:1 *cis*-9	21.81	34.00	63.58
C18:2n-6	23.55	21.66	13.12
C18:3n-3	2.14	1.99	1.46

C control group, *OC* olive cake group

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

[b] Values from NRC (2001)

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, and individual peaks were identified by comparison of their retention indices and mass spectra to those of commercially available standards and mass spectral libraries (NIST) quantitated by peak integration and expressed as a percentage of the total fat.

Statistical analysis

All data were subjected to an analysis using a mixed-effects design with repeated measurements using SAS (version 9.4. SAS Institute Inc., Cary, NC). The model included the fixed effects of time (T), experimental diet (D) and their interaction (T × D), and the random effect of cows and pen. Time was considered a repeated factor, for each analyzed variable. Statistical significance declared at $P < 0.05$. P values between > 0.05 and ≤ 0.10 were interpreted as trending towards significance.

Results

Table 2 shows the DMI, the yield of milk, fat, and protein as well as the fat and protein content of milk during 21 days of forage substitution with ensiled OC. The inclusion of OC in cow diets did not influence neither the DMI, the yield of milk, protein, and fat yield nor the fat and protein percentages in cow milk. The analyzed FA and their aggregated groups determined in milk collected from C and OC treatments are given in Table 3. Feeding ensiled OC significantly increased oleic and total MUFA content, at the expense of a significant decrease of medium-chain FA (MCFA) content in milk. A tendency of decreased levels was demonstrated in total saturated FA ($P < 0.1$), while individual SFA like C4:0 and C16:0 were decreased significantly with OC feeding. In contrast, C18:0 was the only saturated FA that increased by the addition of OC in the cow diets. Among individual 18-C MUFA, increased levels of C18:1 *cis*-9 ($P < 0.05$) and a tendency of elevated of the sum of C18:1 *trans*-10 and C18:1 *trans*-11 were observed in the milk of OC group compared to C group. On the contrary, the contents of C14:1 *cis*-9, C16:1 *cis*-9, and C10:1 *cis*-9 acids were not affected by the inclusion of OC in the cow diets. Although the content of total poly-unsaturated FA (PUFA) was reduced by OC feeding, a tendency ($P = 0.06$) of increased concentration of rumenic acid (CLA *cis*-9, *trans*-11: RA) was observed in the milk of OC group. No differences between treatments were observed in the concentration of α-linolenic (C18:3n-3) and arachidonic (C20:4n-6) acids, while the levels of linoleic acid (C18:2n-6, LA) were reduced by the OC supplementation. The observed FA changes reduced the overall atherogenic index of milk. No diet effect was demonstrated in the desaturation index.

Table 2 Milk production and chemical composition of milk from cows fed diets with 0 or 10 % (DM) of ensiled olive cake during a 21-day feeding trial

	Treatments			*P* value[a]		
Item	Control	OC	SEM	D	T	D × T
DMI, kg/d	22.87	22.90	1.40	0.63	0.51	0.32
Yield, kg/day						
Milk	25.91	25.54	1.93	0.54	0.90	0.20
Fat	0.84	0.86	0.06	0.46	0.71	0.11
Protein	0.91	0.91	0.06	0.35	0.83	†
Milk composition, %						
Fat	3.26	3.41	0.07	0.56	0.49	†
Protein	3.51	3.61	0.06	0.26	0.14	0.24
SnF	9.37	9.39	0.09	0.22	0.35	0.41

C control group, *OC* olive cake group, *SnF* solids non fat

[a] Probability of significant effects due to diet (D), time (T), and their interaction (D × T)

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; †$P < 0.1$: tendency

Discussion

The forage substitution with ensiled OC in the diets of dairy cows for a short-term period, in our study, had a marked effect on milk FA composition by increasing the content of total MUFA and reducing the levels of saturated FA, particularly MCFA. Previously, studies in cows (Castellani et al. 2017; Neofytou et al. 2020), ewes (Chiofalo et al. 2004; Abbeddou et al. 2011a, b, 2015; Symeou et al. 2019, 2021), and goats (Molina-Alcaide et al. 2010) reported a linear decline in SFA content with concomitant increased levels of MUFA by supplemented diets with various forms of processed OC. It is likely that MUFA of feed, escaping rumen biohydrogenation (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 18-C UFA could increase C18:1 isomers in milk arising from ruminal metabolism and from mammary desaturation of C18:0 produced in the rumen (Chilliard et al. 2007). Thus, milk C18:1 *cis*-9 can be elevated either through the action of mammary Δ^9-desaturase with the substrate being stearic acid or by direct transfer from feed (Chilliard et al. 2007). In this regard, since

Table 3 Fatty acid composition (expressed as percentage of total fatty acid methyl esters) of milk from cows fed a diet with 0 or 10 % (DM) of ensiled olive cake during a 21-day feeding trial

Item	Treatments		SEM	*P* value[a]		
	Control	OC		D	T	D × T
C4:0	3.14	2.89	0.1	***	0.99	**
C6:0	1.90	1.90	0.05	0.32	0.12	0.41
C8:0	1.22	1.16	0.04	0.51	†	0.23
C10:0	2.85	2.48	0.16	0.80	*	0.37
C10:1 *cis*-9	0.35	0.31	0.02	0.65	0.16	0.87
C12:0	3.29	2.80	0.17	0.49	0.18	0.90
C14:0	11.19	10.45	0.31	0.23	0.57	0.51
C14:1 *cis*-9	1.37	1.30	0.05	0.23	0.25	0.15
C15:0	1.06	0.80	0.04	0.98	0.11	0.24
C15:0 iso	0.25	0.27	0.02	0.24	0.56	0.26
C16:0	32.64	29.66	0.53	***	0.73	**
C16:1 *cis*-9	1.90	1.66	0.08	0.35	*	0.96
C17:0	0.48	0.43	0.03	0.20	*	0.41
C17:0 ant/iso	0.48	0.41	0.01	0.74	0.79	0.20
C18:0	9.13	11.26	0.43	†	0.81	0.84
C18:1 *trans*-10 + *trans*-11[b]	0.60	0.77	0.08	†	0.64	0.38
C18:1, *cis*-9	19.48	23.16	0.91	*	***	0.61
C18:1, *cis*-11	0.49	0.56	0.04	0.91	***	0.55
C18:2n-6	3.91	3.45	0.20	*	0.89	0.20
C18:3n-3	0.64	0.41	0.07	0.44	0.22	0.74
CLA - *cis*-9, *trans*-11	0.50	0.52	0.03	†	†	0.24
C20:3n-6	0.74	0.64	0.06	†	†	0.20
C20:4n-6	0.95	0.80	0.06	0.26	0.35	0.64
SCFA	9.33	9.11	0.22	0.14	*	†
MCFA	52.22	47.37	0.88	***	0.92	0.33
LCFA	37.41	42.42	1.28	**	**	0.68
<C16	26.76	24.92	0.71	0.98	0.20	0.42
>C16	37.85	42.81	1.29	**	**	0.71
SFA	67.95	65.12	0.75	†	0.26	0.83
MUFA	24.86	28.15	0.93	*	***	0.77
PUFA	7.12	5.89	0.37	*	†	0.20
Atherogenic index	2.64	2.25	0.12	*	*	0.30
Desaturation index	10.95	10.58	0.46	0.21	0.21	0.35

C control group, *OC* olive cake group, *SCFA* short chain fatty acids; C4:0 to C10:1 *cis*-9, *MCFA* medium chain fatty acids; C12:0 to C16:1 *cis*-9, *LCFA* long chain fatty acids; C17:0 to C20:4n-6, atherogenic index = (C12:0 + 4 × C14:0 + C16:0) / (ΣMUFA + ΣPUFA), 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

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

[a] Probability of significant effects due to diet (D), time (T), and their interaction (D × T)

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 of oleic acid and MUFA is related more to the diet rather than to Δ^9-desaturase activity.

Furthermore, the current study showed a reduction in medium-chain saturated FA. This may occur due to either a higher uptake of long-chain FA from the blood, as stated earlier, or/ and a lower de novo synthesis of medium chain FA in the mammary gland (Dorea and Armentano 2017). The de novo FA secretion can be inhibited by bioactive FA, including *trans* CLAs and *trans*-monoenes such as C18:1 *trans*-10 and *trans*-11 (Bauman et al. 2011; Dorea and Armentano 2017) which tended to be elevated in the present study. However, the results of our previous work with

comparable amounts of OC feeding (Neofytou et al. 2020) showed that decreased proportion o*f de novo* FA in milk could occur with no changes in the expression of genes involved in mammary lipid metabolism, suggesting that this mechanism may not play a significant role in the observed data of the current work.

A tendency for increased levels of RA observed in the milk fat of cows fed the OC diet in the present study has been reported previously as a significant increase of RA in studies with cattle (Castellani et al. 2017; Neofytou et al. 2020) and contradictory results observed in sheep fed processed OC (Abbeddou et al. 2011b, 2015). RA can be either synthesized endogenously in the mammary gland through the action of mammary Δ^9-desaturase with the substrate being vaccenic acid, or through the BH of UFA by rumen bacteria such as the *Butyrivibrio fibrisolvens* group (Chilliard et al. 2007; Ferlay et al. 2017). Thus, the existence of difference of RA content in ruminant milk after OC feeding may be attributed to the different process of OC generated, other feed ingredients or different supplementation period. Concerning other UFA, LA concentration in milk was decreased with the supplementation of OC in the cow diets. This is in line with the study of He et al. (2012) who indicated reduced levels of LA after inclusion of high proportion of 18-C UFA in cow diets. In addition, similar results were observed in the milk fat of ewes after the inclusion of olive oil (Gómez-Cortés et al. 2008; Bodas et al. 2010) or processed OC (Abbeddou et al. 2011b, 2015) into their diets. However, previous studies in cows (Castellani et al. 2017; Neofytou et al. 2020) or in small ruminants (Chiofalo et al. 2004; Molina-Alcaide et al. 2010; Tzamaloukas et al. 2021) reported no effect in the concentration of LA in milk fat after OC feeding. The decreased LA levels observed in the milk fat of the OC group could be attributed to the lower LA content contained the OC treatment compared to C diet or to other feeding factors.

The forage substitution with ensiled OC at rates of 10 % in diet DM of lactating cows for a short-term period of 21 days did not affect milk yield as well as the fat and protein content or yield, by using isoenergetic and isonitrogenous diets, similarly to previous studies feeding OC for a long-term period (Hadjipanayiotou 1999; Meo Zilio et al. 2014; Castellani et al. 2017). It has been shown by a meta-analysis study evaluating the effect of adding oil supplements in cow diets 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 conclusion, the use of ensiled OC in the diet of lactating dairy cows, for a short-term period of 21 days, modified the quality of milk by increasing the content of beneficial FA related with positive effects to human health. Those results show that this abundant by-product can be used as a cost-effective forage alternative in cow diets without negative consequences in milk fat content or milk production.

Acknowledgements We would like to thank the technical stuff of the ARI farm for the management of the cows' herd.

Author contribution O.T. conceived and designed research; M.C.N., S.S., and D.S. conducted experiments; D.S. contributed experimental resources; O.T. and D.M contributed laboratory resources and methodology; M.C.N. analyzed data; M.C.N. wrote the manuscript; O.T. and D.M edited and reviewed the manuscript. All authors read and approved the manuscript.

Declarations

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

Informed consent Not applicable to this work.

Conflict of interest The authors declare no competing interests.

References

Abbeddou, S., B. Rischkowsky, M.E.D. Hilali, H.D. Hess, and M. Kreuzer. 2011a. Influence of feeding Mediterranean food industry by-products and forages to Awassi sheep on physicochemical properties of milk, yoghurt and cheese. J. Dairy Res. 78:426–435. doi: https://doi.org/10.1017/S0022029911000665.

Abbeddou, S., B. Rischkowsky, E.K. Richter, H.D. Hess, and M. Kreuzer. 2011b. Modification of milk fatty acid composition by feeding forages and agro-industrial byproducts from dry areas to Awassi sheep. J. Dairy Sci. 94:4657–4668. doi:https://doi.org/10.3168/jds.2011-4154.

Abbeddou, S., B. Rischkowsky, M.E.D. Hilali, M. Haylani, H.D. Hess, and M. Kreuzer. 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. Trop. Anim. Health Prod. 47:145–152. doi:https://doi.org/10.1007/s11250-014-0699-x.

AOAC International. 2005. Official Methods of Analysis. AOAC Int. 18th ed.

Bauman, D.E., K.J. Harvatine, and A.L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299–319. doi:https://doi.org/10.1146/annurev.nutr.012809.104648.

Bodas, R., T. Manso, A.R. Mantecón, M. Juárez, M.Á. De La Fuente, and P. Gómez-Cortés. 2010. Comparison of the fatty acid profiles in cheeses from ewes fed diets supplemented with different plant oils. J. Agric. Food Chem. 58:10493–10502. doi:https://doi.org/10.1021/jf101760u.

Castellani, F., A. Vitali, N. Bernardi, E. Marone, F. Palazzo, L. Grotta, and G. Martino. 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. J. Dairy Sci. 100:8658–8669. doi:https://doi.org/10.3168/jds.2017-12899.

Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828–855. doi: https://doi.org/10.1002/ejlt.200700080.

Chiofalo, B., L. Liotta, A. Zumbo, and V. Chiofalo. 2004. Administration of olive cake for ewe feeding: Effect on milk yield and composition. Small Rumin. Res. 55:169–176. doi:https://doi.org/10.1016/j.smallrumres.2003.12.011.

Costa, E.G.L., F.F. da Silva, R.R. Silva, A.F. Porto, B.M. Santiago, L.C. Rocha, A.G. Cruz, A.C.F. Guedes, T.M. Neto, and E.A. Vieira. 2019. Inclusion of licuri meal in the diet of pasture dairy cows. Trop. Anim. Health Prod. 51:2505–2511. doi:https://doi.org/10.1007/s11250-019-01964-5.

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. 65–68.

Dorea, J.R.R., and L.E. Armentano. 2017. Effects of common dietary fatty acids on milk yield and concentrations of fat and fatty acids in dairy cattle. Pages 2224–2236 in Animal Production Science. CSIRO.

Ferlay, A., L. Bernard, A. Meynadier, and C. Malpuech-Brugère. 2017. Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: a review. Biochimie 141: 107–120. doi:https://doi.org/10.1016/j.biochi.2017.08.006.

Gómez-Cortés, P., P. Frutos, A.R. Mantecón, M. Juárez, M.A. De La Fuente, and G. Hervás. 2008. Addition of olive oil to dairy ewe diets: effect on milk fatty acid profile and animal performance. J. Dairy Sci. 91:3119–3127. doi:https://doi.org/10.3168/jds.2007-0954.

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

He, M., K.L. Perfield, H.B. Green, and L.E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95:1447–1461. doi:https://doi.org/10.3168/jds.2011-4635.

Meo Zilio, D., S. Bartocci, S. Di Giovanni, M. Servili, A. Chiariotti, and S. Terramoccia. 2014. Evaluation of dried stoned olive pomace as supplementation for lactating Holstein cattle: effect on milk production and quality. Anim. Prod. Sci. 55:185–188. doi:https://doi.org/10.1071/AN14254.

Molina-Alcaide, E., E.Y. Morales-García, A.I. Martín-García, H. Ben Salem, A. Nefzaoui, and M.R. Sanz-Sampelayo. 2010. Effects of partial replacement of concentrate with feed blocks on nutrient utilization, microbial N flow, and milk yield and composition in goats. J. Dairy Sci. 93:2076–2087. doi:https://doi.org/10.3168/jds.2009-2628.

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

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed, National Academy of Sciences, Washington, DC.

Shingfield, K.J., L. Bernard, C. Leroux, and Y. Chilliard. 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140–1166. doi:https://doi.org/10.1017/s1751731110000510.

Symeou, S., C.G. Tsiafoulis, I.P. Gerothanassis, D. Miltiadou, and O. Tzamaloukas. 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 Rumin. Res. 177:111–116. doi:https://doi.org/10.1016/j.smallrumres.2019.06.017.

Symeou, S., O. Tzamaloukas, G. Banos, and D. Miltiadou. 2020. ACAA2 and FASN polymorphisms affect the fatty acid profile of Chios sheep milk. J. Dairy Res.. doi:https://doi.org/10.1017/S0022029919000992.

Symeou, S., D. Miltiadou, C. Constantinou, P. Papademas, and O. Tzamaloukas 2021. Feeding olive cake silage up to 20 percent of DM intake in sheep improves lipids quality and health related indices of milk and ovine Halloumi cheese Trop. Anim. Health and Prod. (in press)

Tzamaloukas, O., M. Orford, D. Miltiadou, and C. Papachristoforou. 2015. Partial suckling of lambs reduced the linoleic and conjugated linoleic acid contents of marketable milk in Chios ewes. J. Dairy Sci. 98:1739–1749. doi:https://doi.org/10.3168/jds.2014-8540.

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

Van-Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:https://doi.org/10.3168/jds.S0022-0302(91)78551-2.