Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats

Article *in* animal · April 2017

DOI: 10.1017/S175173111700060X

CITATIONS
0

READS
23

8 authors, including:

Zeljka Klir
University of Osijek
19 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Boro Mioč
University of Zagreb
269 PUBLICATIONS **444** CITATIONS
SEE PROFILE

Uta Dickhoefer
Hohenheim University
39 PUBLICATIONS **252** CITATIONS
SEE PROFILE

Antunović Zvonko
University of Osijek
110 PUBLICATIONS **293** CITATIONS
SEE PROFILE

Animal (2017), **11:10**, pp 1772–1778 © The Animal Consortium 2017
doi:10.1017/S175173111700060X

animal

Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats

Z. Klir[1], J. M. Castro-Montoya[2], J. Novoselec[1], J. Molkentin[3], M. Domacinovic[1], B. Mioc[4], U. Dickhoefer[2†] and Z. Antunovic[1]

[1]*Department for Animal Husbandry, Faculty of Agriculture in Osijek, University of J. J. Strossmayer, V. Preloga 1, 31000 Osijek, Croatia;* [2]*Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Fruwirthstrasse 31, 70593 Stuttgart, Germany;* [3]*Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institute, Hermann-Weigmann-Strasse 1, 24103 Kiel, Germany;* [4]*Department of Animal Science and Technology, Faculty of Agriculture, University of Zagreb, Svetosimunska cesta 25, 10000 Zagreb, Croatia*

(Received 25 June 2016; Accepted 25 February 2017; First published online 3 April 2017)

The aim was to determine the effect of substituting pumpkin seed cake (PSC) or extruded linseed (ELS) for soya bean meal in goats' diets on milk yield, milk composition and fatty acids profile of milk fat. In total, 28 dairy goats were divided into three groups. They were fed with concentrate mixtures containing soya bean meal (Control; n *= 9), ELS (*n *= 10) or PSC (*n *= 9) as main protein sources in the trial lasting 75 days. Addition of ELS or PSC did not influence milk yield and milk gross composition in contrast to fatty acid profile compared with Control. Supplementation of ELS resulted in greater branched-chain fatty acids (BCFA) and total n-3 fatty acids compared with Control and PSC (*P *< 0.05). Total n-3 fatty acids were accompanied by increased α-linolenic acid (ALA, C18:3n-3; 0.56 g/100 g fatty acids) and EPA (C20:5n-3; 0.12 g/100 g fatty acids) proportions in milk of the ELS group. In contrast, ELS and PSC resulted in lower linoleic acid (LA, C18:2n-6; 2.10 and 2.28 g/100 g fatty acids, respectively) proportions compared with Control (2.80 g/100 g fatty acids;* P *< 0.05). Abovementioned resulted in lower LA/ALA ratio (3.81* v. *7.44 or 6.92, respectively;* P *< 0.05) with supplementation of ELS compared with Control or PSC. The PSC diet decreased total n-6 fatty acids compared with the Control (2.96* v. *3.54 g/100 g fatty acids,* P *< 0.05). Oleic acid (*c9-C18:1*), CLA (*c9,t11-18:2*) and* t10-,t11-C18:1 *did not differ between treatments (*P ⩾ *0.08), although stearic acid (C18:0) increased in ELS diets compared with Control (12.7* v. *10.2 g/100 g fatty acids,* P *< 0.05). Partially substituted soya bean meal with ELS in hay-based diets may increase beneficial n-3 fatty acids and BCFA accompanied by lowering LA/ALA ratio and increased C18:0. Pumpkin seed cake completely substituted soya bean meal in the diet of dairy goats without any decrease in milk production or sharp changes in fatty acid profile that may have a commercial or a human health relevancy.*

Keywords: fatty acid profile, extruded linseed, pumpkin seed cake, goat milk

Implications

To our knowledge this is the first study showing the inclusion of pumpkin seed cake (PSC) in the goats' diets. Pumpkin seed cake was successfully used as replacement for soya bean meal in the diet of dairy goats without any negative effect on milk yield or milk composition. Similarly, the current study confirmed the beneficial role of extruded linseed (ELS) in increasing the n-3 fatty acids content in goat milk, a feature desired by certain groups of consumers. The effects of PSC supplementation on profitability and environmental issues like nitrogen excretions should still be evaluated.

† E-mail: uta.dickhoefer@uni-hohenheim.de

Introduction

Numerous investigations have reported differences in the role of fatty acids for human health, depending on their chain length, number and position of double bonds, and their configuration and ratio of certain fatty acids. The effects of individual fatty acids consumption is a complex issue to study provided that fatty acids cannot be longer viewed as general categories (Vannice and Rasmussen, 2014), but in general high intake of certain saturated and *trans*-fatty acids and low intake of polyunsaturated fatty acids (PUFA) are associated with increased cardiovascular disease risk (Mozaffarian *et al.*, 2006; Siri-Tarino *et al.*, 2010).

Fat supplementation in the diets of animals does not only enhance the energy content of the diet, but also has the

potential to modify the fatty acid profile of animal products, depending on proportions of fatty acids in the animals' diets, the level of biohydrogenation and the rumen microbial activity (Mele *et al.*, 2008). Milk fat in ruminants contains ~400 different fatty acids which makes it the most complex of all natural fats (Månsson, 2008) and presents a paramount factor in the nutritional quality of cheeses (Bodas *et al.*, 2010). Goats' milk contains a higher content of certain short-chain fatty acids (C6:0, C8:0, C10:0) than cows' milk which contribute to better digestibility of fat in their milk or milk products by humans (Queiroga *et al.*, 2013).

By-products from oil production are used in animal nutrition to meet energy and protein requirements of ruminants. High and variable soya bean prices has led to attempt a partial or total replacement of soya bean as protein source for ruminants with alternative feedstuffs (Vasta *et al.*, 2008). In this regard, PSC is a by-product with potential for ruminants' feeding, with a higher CP concentration than soya bean meal (Zdunczyk *et al.*, 1999) which effects on goats' milk yield and composition have not been studied. The main fatty acids in the fat of PSC are linoleic acid (LA, 35.6 to 60.8 g/100 g fat), oleic acid (OA, 21.0 to 46.9 g/100 g fat), palmitic acid (9.5 to 14.5 g/100 g fat) and stearic acid (3.1 to 7.4 g/100 g fat) (Murković *et al.*, 1996). Similarly, ELS is one of the most common by-products available and already adopted for enrichment of dairy products with beneficial fatty acids. Linseed is rich source of unsaturated fatty acids (UFA), particularly α-linolenic acid (ALA), which concentration in fat is 51.3 g/100 g (Bodas *et al.*, 2010). The research by Nudda *et al.* (2006) indicated high-nutritional value of ELS, which leads to an increase in ALA, CLA and vaccenic acid. The aim of this study was to research if PSC inclusion in the diets of dairy goats influences yield, composition and fatty acids profile of their milk as compared with the feeding of a conventional diet containing soya bean meal and a diet already replacing some soya bean meal with ELS as protein source.

Material and methods

Experimental design, animals and feeding

The research was conducted with 28 French Alpine dairy goats at the commercial farm Đurković in Marjančaci (Croatia). All goats had kidded within a period of 2 weeks in February 2014 and were included in the experiment on the 5th of March. The total duration of the experiment was 75 days and was conducted between March and May 2014. All goats were healthy and in good condition when the experiment started, and had an average age of 43.2 ± 20.4 months and an average live weight of 46.9 ± 7.9 kg (Table 1). All goats were fed *ad libitum* (~2.5 kg/goat per day) with a basal diet of a mixture of grass (600 g/kg dry matter (DM); *Lolium multiflorum* and *Phleum pratense*) and red clover hay (400 g/kg DM; *Trifolium pratense*). They were divided into three groups. In addition to the basal diet, each goat was individually supplemented with 1 kg/day of a concentrate mixture, in separated feeding troughs, offered in two portions during the morning and evening milking, no refusals of concentrate were observed for any animal. The concentrate mixture differed in the main source of protein (Table 2)

1. Control group ($n = 9$): soya bean meal as main protein source.
2. Extruded linseed group ($n = 10$): soya bean meal was partially substituted with ELS.
3. Pumpkin seed cake group ($n = 9$): soya bean meal was completely substituted with PSC.

Concentrate mixtures were isoproteic, isolipidic and isoenergetic (National Research Council, 2007). The diet had an

Table 1 *Experimental design and composition (as-fed) of experimental diets offered to lactating dairy goats (arithmetic mean ± SD)*

	Diets		
Parameters	Control	ELS	PSC
Goats (*n*)	9	10	9
Age (months)	33.3 ± 5.29	44.4 ± 13.91	52.0 ± 31.75
BW (kg)	46.9 ± 6.84	46.8 ± 9.18	46.9 ± 8.37
Feeding			
Concentrate mixture	1 kg/head per day of Control	1 kg/head per day of ELS	1 kg/head per day of PSC
Grass–clover hay[1]	*Ad libitum*	*Ad libitum*	*Ad libitum*

ELS = extruded linseed; PSC = pumpkin seed cake.
[1]Hay mixture contained (on dry matter basis) 60% of *Phleum pratense* and *Lolium multiflorum* hay and 40% of *Trifolium pratense* hay.

Table 2 *Ingredient composition (g/kg, as-fed) of the concentrate mixtures containing soya bean meal (Control), extruded linseed (ELS) or pumpkin seed cake (PSC) in the diets of lactating dairy goats*

	Concentrate mixtures		
Ingredients	Control	ELS	PSC
Corn	429	408	459
Barley	80	80	90
Oats	100	100	135
Wheat flour	120	90	120
Extruded soya bean meal	150	–	–
ELS	–	90	–
PSC	–	–	160
Alfalfa dehydrated	–	40	–
Soya bean meal (46% CP)	85	157	–
Calcium carbonate	16	15	16
Monocalcium phosphate	5	5	5
Salt	4	4	4
Pellet binder	1	1	1
Premix[1]	10	10	10

[1]Mineral–vitamin premix: Fe (iron sulphate monohydrate) 4000 mg, Cu (copper sulphate pentahydrate) 800 mg, Mn (manganese oxide) 3500 mg, Zn (zinc sulphate monohydrate) 5000 mg, I (potassium iodide) 80 mg, Co (cobalt sulphate heptahydrate) 20 mg, Se (sodium selenite) 15 mg, Mg (magnesium oxide) 5000 mg; vitamin A 0.1×10^7 IU, vitamin D_3 150 000 IU, vitamin E (α-tocopherol) 1500 mg, vitamin K_3 (menadione sodium bisulfite) 50 mg, vitamin B_1 (thiamine mononitrate) 100 mg, vitamin B_2 200 mg, vitamin B_6 200 mg, vitamin B_{12} 1 mg, niacin 1000 mg, Ca-pantothenate 500 mg, choline chloride 10 000 mg.

https://doi.org/10.1017/S175173111700060X

approximate hay to concentrate ratio of 70 : 30 (on DM basis) with 18.12 MJ net energy for lactation (NE_L)/day with ~40.5% of total NE_L/day derived from the concentrate. All animals had free access to fresh drinking water. During the 1st month after kidding, kids were kept together with the goats and were allowed to suckle *ad libitum*. Kids were completely weaned at the age of 2 months, and kept separately from their mothers thereafter.

Chemical composition of feedstuffs

Four replicates of ~10 g of each concentrate mixture, PSC, ELS and hay mixture, were sampled, dried at 60°C, ground to 1 mm particle size using a cutting mill (Microtron MB 550; Kinematica, Luzern, Switzerland) and analysed for chemical composition (Table 3). In brief, crude ash (CA) concentrations were determined by incinerating the feed samples at 550°C for 6 h. The CP concentrations were estimated from nitrogen content according to the Kjeldahl method (Pearson, 1976). Crude lipids concentrations were analysed according to Onwuka (2005) using the universal Extraction System B-811 (Buchi, Flawil, Switzerland). Finally, crude fibre concentrations were determined using the Weende method described in Offor *et al.* (2014).

Metabolisable energy (MJ/kg DM) and digestibility of organic matter (g/kg DM) of feed samples were calculated from gas production after 24 h of *in vitro* incubation with Hohenheim gas test using the following equations (Menke *et al.*, 1979)

$$\mathrm{ME\,(MJ/kg\,DM)} = 1.06 + 0.157 \times \mathrm{Gp} + 0.0084 \times \mathrm{CP} + 0.022 \times \mathrm{CL} - 0.0081 \times \mathrm{CA} - \text{for hay}, \quad (1)$$

$$\mathrm{ME\,(MJ/kg\,DM)} = 2.20 + 0.136 \times \mathrm{Gp} + 0.0057 \times \mathrm{CP} + 0.00029 \times \mathrm{CL}^2 - \text{for concentrates}, \quad (2)$$

$$\mathrm{DOM\,(g/kg\,DM)} = 149 + 8.89 \times \mathrm{Gp} + 0.448 \times \mathrm{CP} + 0.651 \times \mathrm{CA} - \text{for hay and concentrates}, \quad (3)$$

where DM is the dry matter (g/kg fresh matter), Gp the gas production (ml/200 mg DM), CP the crude protein concentration (g/kg DM), CL the crude lipids concentration (g/kg DM) and CA the crude ash concentration (g/kg DM).

Fatty acid analysis of feedstuffs

Fatty acids in dietary ingredients were determined by gas chromatography following the methodology of State office of agricultural chemistry in Baden Württemberg (LaChemie P23-5-008, V. 01, Determination of the complete fatty acids profile as fatty acids methyl esters in dietary compounds). Fatty acid proportions of concentrate mixtures are presented in Table 4.

Milk sampling

Goats were hand-milked twice a day at 0700 and 1900 h. Milk samples were taken on the 20th, 48th and 75th days after starting the experiment, however, during the first two sampling kids were still suckling and therefore only the analyses of samples taken on day 75 are presented here. For the fatty acid analysis four 100-ml sub-samples were taken as follows: evening milking on day 74, morning and evening on day 75 and morning milking on day 76. The four sub-samples were pooled and weighted by milk production to

Table 3 *Chemical composition of hay (n = 4), pumpkin seed cake (PSC; n = 4), extruded linseed (ELS; n = 4) and the concentrate mixtures used in the Control, ELS and PSC diets of lactating dairy goats (arithmetic means)*

	Concentrate mixtures					
Parameters	Control	ELS	PSC	Hay	ELS	PSC
DM (g/kg fresh matter)	876	874	873	925	924	939
CP (g/kg DM)	162	162	163	110	228	529
Crude fibre (g/kg DM)	41.4	48.8	37.3	287	18.8	39.4
Crude ash (g/kg DM)	49.2	50.6	52.3	57.3	35.2	85.1
Crude fat (g/kg DM)	56.4	58.3	56.3	13.3	260	163
ME (MJ/kg DM)[1]	13.2	13.0	13.2	8.0	13.7	13.1
DOM (g/kg DM)[2]	819	806	822	572	571	654

DM = dry matter; ME = metabolisable energy; DOM = digestibility of organic matter; Gp = gas production (ml/200 mg DM); CP = crude protein concentration (g/kg DM); CL = crude lipids concentration (g/kg DM); CA = crude ash content (g/kg DM).
[1]ME (MJ/kg DM) = 1.06 + 0.157 × Gp + 0.0084 × CP + 0.022 × CL − 0.0081 × CA (equation for hay); ME (MJ/kg DM) = 2.20 + 0.136 × Gp + 0.0057 × CP + 0.00029 × CL^2 (equation for concentrates).
[2]DOM (g/kg DM) = 149 + 8.89 × Gp + 0.448 × CP + 0.651 × CA (for hay and concentrates) (Menke *et al.*, 1979).

Table 4 *Proportions (g/100 g fatty acids) of fatty acids in hay and concentrate mixtures containing soya bean meal (Control), extruded linseed (ELS) or pumpkin seed cake (PSC) in the diets of lactating dairy goats*

	Concentrate mixtures			
Fatty acids	Control	ELS	PSC	Hay
C6:0	0.05	0.06	0.08	0.28
C8:0	0.02	0.07	0.03	0.17
C10:0	0.03	0.11	0.02	0.19
C11:0	ND	ND	ND	0.05
C12:0	0.03	0.12	0.03	0.56
C13:0	ND	ND	ND	0.07
C14:0	0.15	0.71	0.15	1.52
C15:0	0.06	ND	ND	0.80
C16:0	10.7	9.43	12.9	29.8
*c*9-C16:1	0.20	0.15	0.17	0.64
C17:0	0.15	0.19	0.13	0.39
C18:0	4.08	6.11	4.48	2.84
*c*9-C18:1	31.9	32.0	34.4	7.71
C18:2n-6	48.2	38.2	44.4	22.4
C18:3n-3	2.97	11.50	1.80	24.5
C20:0	ND	ND	ND	0.84
C20:4n-6	0.29	0.56	0.30	ND
C21:0	ND	0.42	ND	ND
C22:0	0.42	0.39	0.42	1.71
C23:0	0.30	ND	0.48	3.60

ND = not determined.

https://doi.org/10.1017/S175173111700060X

one sample. Milk yield of each goat was recorded with a measuring cylinder from these samplings. Milk yield expressed in volume (l) was converted in mass (kg) with the conversion factor 1.032 (normal goat milk density) according to International Committee for Animal Recording (2012).

Analysis of milk composition

Immediately after sampling the milk was transferred into 100-ml bottles containing 0.3 ml of the preservative azidiol, cooled to 4°C and transported to the Central Laboratory for Milk Control in Križevci (Croatia) for the analysis of fat (g/100 g), protein (g/100 g), lactose (g/100 g), urea (mg/dl) and somatic cell counts (SCC) (*n*/ml). Composition was analysed by IR spectroscopy HR ISO 92622:2001 method on the analyser MilkoScan FT 6000 (Foss Electric, Hillerød, Denmark). Somatic cell counts were determined by fluoro-opto-electronic method HR ISO 13366-2/Ispr.1:2007 with a Fossomatic 5000 analyser (Foss Electric).

Fatty acid analysis

After sampling milk was frozen at −80°C and stored until analysis. The extraction of milk fat was done following a three-step extraction with gravimetric reference method (The International Organization for Standardization, 2010). Fatty acid methyl esters (FAME) were obtained from the milk fat samples by transesterification according to The International Organization for Standardization (2002a), whereas the fatty acid composition was determined by GLC following procedures described in The International Organization for Standardization (2002b).

In brief, a mixture of 1200 µl of n-heptane, 300 µl of a 10% solution of fat in n-heptane and 60 µl of a 2 mol/l solution of potassium hydroxide in methanol was shaken vigorously for 2 min (vortex mixer) in a sample vial and allowed to stand for 5 min. After addition of 150 mg sodium hydrogen sulfate monohydrate the sample was vortexed (1 min) and centrifuged (3 min at 1000 × **g**); 1 µl of the supernatant (~2% FAME in heptane) was injected into the gas chromatograph (Agilent 7890A; Agilent, Santa Clara, CA, USA). The gas chromatograph was equipped with an Agilent 7683B autosampler (Agilent), a split injection port (1:130), a flame ionisation detector and a 60-m fused silica capillary column (internal diameter 0.25 mm) coated with a 0.20 µm film of CP-Sil 88 (Agilent). Hydrogen was used as carrier gas at a constant flow of 1.3 ml/min (94 kPa initial pressure). Injector and detector temperatures were 255°C. The oven temperature was maintained at 50°C, isothermal, for 1 min then programmed at 5°C/min to 225°C, which was held for 3 min isothermal, and then programmed at 1°C/min to 237°C. Evaluation of chromatograms was performed with the Agilent Software EZChrom Elite 3.3.2. Calibration of the major fatty acids was carried out using the reference milk fat CRM 164 (IRMM, Geel, Belgium). Fatty acids in the range from C4 to C24 were determined, calculated as weight percentage (g/100 g fatty acids) and are presented as means of duplicate analyses.

Table 5 *Yield and composition of milk of dairy goats offered concentrate mixtures containing soya bean meal (Control;* n = *9), extruded linseed (ELS;* n = *10) or pumpkin seed cake (PSC;* n = *9) (arithmetic means)*

	Diets				
Parameters	Control	ELS	PSC	SEM	*P*-values
Milk yield (kg/milking[1])	1.09	1.11	1.25	0.06	0.62
Milk composition (g/100 g)					
Fat	3.22	3.50	3.12	0.08	0.23
Protein	2.72	3.01	2.76	0.06	0.07
Lactose	4.28	4.38	4.31	0.03	0.23
Urea (mg/dl)	37.1	32.8	36.5	1.15	0.28
Somatic cells count (log n/ml)	5.91	5.73	5.87	0.10	0.76

[1]Morning milking.

Statistical analysis

The results are presented as arithmetic means and standard error of mean. Data were analysed using GLM procedure (SAS 9.3®) with diet treatment as fixed effect and parity, litter size as covariate for milk yield and composition analyses, whereas fatty acid proportion was analysed using milk yield as additional covariate. Means were compared using the Tukey's test and differences between the groups were declared significant at $P < 0.05$. Values of SCC were logarithmically converted to a linear score with the aim to approximate normal distribution.

Results and discussion

Milk yield and composition

To the best of our knowledge, there is no scientific literature available on the effects of PSC feeding of goats on milk yield and composition. The contents of protein, fat, lactose, urea and the SCC in milk did not differ between ELS or PSC and the Control ($P \geqslant 0.07$; Table 5). Nudda *et al.* (2006) found no effects of ELS cake on milk yield and composition. Neither linseed oil in the study by Martínez-Marín *et al.* (2012) had effect on milk production traits of goats.

The inclusion of ELS or PSC as substitute of soya bean meal might have altered voluntary hay intake of goats in our study, which could not be measured under the current experimental setup. However, studies with sheep supplemented with fat in the form of seeds (Zhang *et al.*, 2007) or oils (Ivan *et al.*, 2001) did not report negative effects on DM intake. Along the same line, Allen (2000) found that the response in DM intake of dairy cows to fat supplementation depends on the fat content of the initial diet, with the strongest effects occurring when fat is actually added and not substituted by another source as in this study. Therefore, as chemical composition of the three concentrate mixtures was similar (Table 3) and they were consumed at the same rate (1 kg/animal), ELS and PSC feeding likely did not pronouncedly alter hay intake of goats. Overall, our results thus show the potential of replacing soya bean meal partially by ELS or completely by PSC in the diets of dairy goat without any compromises in milk yield and composition.

https://doi.org/10.1017/S175173111700060X

Table 6 *Proportions (g/100 g fatty acids) of fatty acids in milk of dairy goats offered concentrate mixtures containing soya bean meal (Control;* n = *9), extruded linseed (ELS;* n = *10) or pumpkin seed cake (PSC;* n = *9) (arithmetic means)*

	Diets				
Fatty acids	Control	ELS	PSC	SEM	*P*-value
C4:0	2.52	2.51	2.63	0.04	0.36
C5:0	0.02	0.02	0.02	0.001	0.38
C6:0	2.58	2.56	2.64	0.04	0.45
C7:0	0.03	0.04	0.03	0.001	0.28
C8:0	2.70	2.75	2.75	0.06	0.65
C9:0	0.06	0.07	0.06	0.003	0.28
C10:0	9.14	8.85	9.18	0.21	0.75
C10:1	0.17	0.17	0.18	0.01	0.94
C11:0	0.08	0.09	0.08	0.005	0.43
C12:0	3.67	3.47	3.56	0.11	0.87
C12:1	0.06	0.06	0.05	0.002	0.93
iso-C13	0.02^{b}	0.04^{a}	0.03^{a}	0.002	0.003
aiso-C13	0.01	0.01	0.01	0.001	0.67
C13:0	0.09	0.10	0.08	0.002	0.08
iso-C14	0.09	0.11	0.10	0.004	0.26
C14:0	9.35^{a}	8.45^{b}	9.28^{a}	0.15	0.01
*c*9-C14:1	0.10	0.09	0.10	0.004	0.71
iso-C15	0.20^{b}	0.25^{a}	0.22^{ab}	0.01	0.04
aiso-C15	0.35^{b}	0.43^{a}	0.38^{ab}	0.01	0.009
C15:0	0.80^{b}	0.89^{a}	0.84^{ab}	0.02	0.04
iso-C16	0.24^{b}	0.30^{a}	0.27^{ab}	0.01	0.008
C16:0	25.7^{a}	22.2^{b}	25.8^{a}	0.55	0.002
*t*9-C16:1	0.33	0.34	0.32	0.01	0.50
*c*9-C16:1	0.43	0.40	0.42	0.01	0.54
iso-C17	0.44	0.50	0.44	0.01	0.05
aiso-C17	0.46^{ab}	0.52^{a}	0.44^{b}	0.01	0.03
C17:0	0.64	0.67	0.61	0.01	0.05
C17:1	0.17	0.18	0.16	0.01	0.58
iso-C18	0.05	0.06	0.05	0.002	0.05
C18:0	10.2^{b}	12.7^{a}	11.2^{ab}	0.34	0.009
*t*4-C18:1	0.02	0.03	0.02	0.001	0.05
*t*5-C18:1	0.02	0.03	0.02	0.001	0.23
*t*6-8-C18:1	0.30	0.30	0.27	0.01	0.54
*t*9-C18:1	0.29	0.31	0.27	0.01	0.10
*t*10-11-C18:1	2.66	2.54	2.17	0.11	0.54
*t*15-C18:1	0.20^{b}	0.27^{a}	0.20^{b}	0.01	<0.001
*c*9-C18:1	16.8	18.6	17.3	0.34	0.08
*c*11-C18:1	0.34	0.33	0.30	0.01	0.22
*c*12-C18:1	0.40	0.38	0.31	0.02	0.56
*c*13-C18:1	0.08	0.08	0.07	0.002	0.24
*t*16 + *c*14-C18:1	0.35^{b}	0.44^{a}	0.34^{b}	0.35	<0.001
*c*15 + C19-C18:1	0.19^{b}	0.26^{a}	0.19^{b}	0.01	<0.001
C18:2n-6	2.80^{a}	2.10^{b}	2.28^{b}	0.08	0.003
*c*9,*t*11-C18:2	0.96	0.97	0.81	0.04	0.39
*t*9,*t*12 + *t*8,*c*12 + *c*9, *t*13-C18:2	0.35	0.44	0.33	0.01	0.002
*t*8,*c*13-C18:2	0.18^{ab}	0.21^{a}	0.16^{b}	0.01	0.02
*c*9,*t*12-C18:2	0.10	0.10	0.08	0.004	0.33
*t*11,*c*15-C18:2	0.14^{b}	0.32^{a}	0.13^{b}	0.02	<0.001
C18:3n-6	0.08^{a}	0.02^{ab}	0.02^{b}	0.001	0.01
C18:3n-3	0.38^{b}	0.56^{a}	0.33^{b}	0.02	<0.001
C19:1	0.02^{b}	0.03^{a}	0.02^{b}	0.001	0.01
C19:2	0.04	0.04	0.04	0.002	0.69
C20:0	0.26	0.28	0.26	0.01	0.16
C20:1	0.05	0.05	0.05	0.001	0.81
C20:2	0.03	0.03	0.02	0.001	0.06
C20:3	0.03^{b}	0.06^{a}	0.04^{ab}	0.004	0.01
C20:4n-6	0.20^{a}	0.16^{b}	0.18^{ab}	0.01	0.01
C20:5n-3	0.09^{b}	0.12^{a}	0.09^{b}	0.004	<0.001
C21:0	0.06	0.06	0.05	0.002	0.21
C22:0	0.07^{ab}	0.09^{a}	0.07^{b}	0.003	0.02
C22:1	0.01	0.01	0.01	0.001	0.12
C22:5n-3	0.15^{ab}	0.17^{a}	0.13^{b}	0.01	0.008
C23:1	0.04	0.04	0.04	0.001	0.62
C24:0	0.01	0.01	0.01	0.0005	0.10

^{a,b}Values within a row with different superscript letters differ significantly at $P < 0.05$.

Milk fatty acid profile

The ELS and PSC diets did not influence the proportions of C6:0, C8:0 and C10:0 ($P \geqslant 0.45$; Table 6), which is desirable, because these fatty acids are responsible for the particular sensory properties of goat's cheese upon lipolysis. Similar results were observed by Nudda *et al.* (2006) who offered a diet providing 100 g/day per animal of ELS to dairy goats over a period of 21 days. Chilliard *et al.* (2003) stated that *de novo* synthesised fatty acid proportions may decrease, if biohydrogenation of dietary UFA increases C18:0 and *t*11-C18:1 proportions, as long-chain fatty acids (LCFA) inhibit lipogenic enzymes of the mammary gland (Mele *et al.*, 2008).

For medium-chain fatty acids, ELS had lower C14:0 and C16:0 concentrations than both PSC and Control. The lower C16:0 in ELS compared with Control is in agreement with results of previous studies (Nudda *et al.*, 2006 and 2008; Renna *et al.*, 2013). A proportion of 40% of C16:0 in milk originates from *de novo* synthesis in the mammary gland and another 60% is absorbed from circulation (Chilliard and Ferlay, 2004; Nudda *et al.*, 2006). In the present study both PSC and Control had higher C16:0 concentrations than ELS (Table 4). In addition, the decrease of C16:0 in ELS group, could be also due to increased proportion of LCFA which may inhibit *de novo* synthesis of fatty acids as suggested by Chilliard *et al.* (2003). Stearic acid (C18:0) proportion increased with addition of ELS compared with the Control, likely due to higher rate of complete biohydrogenation of dietary PUFA that could have been biohydrogenated to C18:0, whereas C18:0 concentration in milk fat of PSC was in an intermediate concentration between control and ELS. The sum of saturated fatty acids was not affected by ELS and PSC (Table 7; $P = 0.06$).

Total odd and branched-chain fatty acids (OBCFA; $P \leqslant 0.01$) and some individual BCFA (iso-C15, aiso-C15 and iso-C16; $P \leqslant 0.04$) were higher in ELS compared with the Control, with the PSC treatment being in most cases intermediate between the former two. As these fatty acids derive

https://doi.org/10.1017/S175173111700060X

Table 7 *Proportions (g/100 g fatty acids) of groups of fatty acid in milk of dairy goats offered concentrate mixtures containing soya bean meal (Control;* n = *9), extruded linseed (ELS;* n = *10) or pumpkin seed cake (PSC;* n = *9) (arithmetic means)*

	Diets				
Fatty acids	Control	ELS	PSC	SEM	*P*-value
SCFA	17.2	17.0	17.5	0.30	0.63
MCFA	41.5[a]	37.2[b]	41.6[a]	0.63	0.002
LCFA	39.7[b]	44.2[a]	39.5[b]	0.73	0.01
SFA	69.9	68.0	71.1	0.53	0.06
BCFA	1.87[b]	2.22[a]	1.94[b]	0.05	0.008
OFA	2.05[b]	2.24[a]	2.04[b]	0.03	0.01
∑C16:1 isomers	0.76	0.74	0.74	0.02	0.86
∑C18:1 isomers	2.21[ab]	2.42[a]	2.00[b]	0.06	0.03
∑C18:2 isomers	0.77[b]	1.07[a]	0.71[b]	0.04	<0.001
MUFA	23.9	25.7	23.5	0.42	0.07
UFA	29.4	31.1	28.2	0.51	0.08
PUFA	5.49	5.32	4.65	0.15	0.18
∑n-3	0.62[b]	0.86[a]	0.56[b]	0.03	<0.001
∑n-6	3.54[a]	2.92[b]	2.96[b]	0.09	0.03
LA/ALA	7.44[a]	3.81[b]	6.92[a]	0.35	<0.001

SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; BCFA = branched-chain fatty acids; OFA = fatty acids with odd number of carbon atoms; MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids; LA = linoleic acid; ALA = α-linolenic acid.
[a,b]Values within a row with different superscript letters differ significantly at $P < 0.05$.

from bacteria living in the rumen, higher proportions of OBCFA with ELS diets might indicate an increase in growth and/or a shift in the species composition of the microbial consortium in the rumen (Vlaeminck *et al*., 2006); Mika *et al*. (2016) reported anti-inflammatory, antidiabetic and anticancer properties of iso-BCFA, and potential benefits of iso-BCFA enriched diets. In contrast to our results, previous studies showed a decrease in content of BCFA in milk when linseed oil (Martínez-Marín *et al*., 2011) or formaldehyde-treated linseed (Bernard *et al*., 2005) were added to the diets of lactating goats, which has been also observed when supplemented diets with fat containing high amounts of either LA, ALA or EPA and DHA (Vlaeminck *et al*., 2006).

Although proportions of total C18:1 isomers increased in ELS, compared with PSC, proportions of OA and *t*10-,*t*11-C18:1 were not different between these two treatments. Total monounsaturated fatty acid proportion was not affected by ELS and PSC ($P = 0.07$). Similar findings were reported by Chilliard *et al*. (2003) who supplemented dairy goats with 3.4 ± 0.6% (DM basis) added lipids as crude whole linseed *v*. the group supplemented with lipids as soya beans in the same concentration. The proportion of C18:1 may be the result of the desaturation of C18:0 available in mammary gland which, in turn, originates from the biohydrogenation of PUFA in the rumen (Chilliard and Ferlay, 2004).

Diets with PSC decreased ($P < 0.05$) LA by 18.6% and with ELS by 25.0% compared with the Control likely as consequence of the higher LA content of soya bean meal included in the Control diet (Table 4) which is in agreement with previous findings of Chilliard *et al*. (2003) and Renna *et al*. (2013).

Isomers *t*15-C18:1 and *t*11,*c*15-C18:2, both produced from ALA metabolism in rumen (Shingfield *et al*., 2010), were higher in ELS than in Control group of the present study. Contrary, PSC diet lowered proportions of ALA compared with ELS diet in accordance with lower *t*15-C18:1 and *t*11,*c*15-C18:2. As isomers of LA in milk are intermediates of biohydrogenation of LA and ALA (Shingfield *et al*., 2010), total C18:2 isomers were also higher with ELS than with Control or PSC diets. Furthermore, CLA (*c*9,*t*11-C18:2), one of the milk fatty acids of interest with respect to human health, was not affected by ELS or PSC ($P > 0.05$).

Proportions of arachidonic acid (AA, C20:4n-6) were lower (20%) in ELS, likely due to lower proportion of LA in the diet (Table 4), whereas PSC did not influence AA proportion. Conversely, ELS increased ALA proportions (47.4%) compared with Control group ($P < 0.05$), but no effects were observed with PSC. Similar to findings of Nudda *et al*. (2008) and Renna *et al*. (2013), proportions of AA formed were lower in milk of goats of the ELS group than of those from Control group may be due to lower availability of LA as a precursor. Content of ALA was highest in the ELS diets (Table 4.) which resulted in higher ALA proportions in milk of goats of the ELS group compared with those of the Control or PSC group. Consequently, the LA/ALA ratio was lowest in the ELS group which is common observation when ELS is added in goat diets, also concluded by Renna *et al*. (2013). However, LA/ALA ratio in milk did not differ between goats of the PSC and Control group. Although n-6/n-3 ratio may have been misguided regarding cardiovascular disease (Salter, 2013) there are many diseases that may develop due to imbalance of LA/ALA (Simopoulos, 2011).

Proportion of EPA in milk fat was increased (33.3%; $P < 0.05$) with ELS diet, as a consequence of greater ALA supply from the diet as its precursor during the elongation. However, milk fat proportion of docosapentaenoic acid (DPA) was not different between ELS and Control diets. The PSC diet did not affect EPA compared with Control, but lowered DPA compared with ELS. Total n-3 fatty acids were by 27.9% higher in the ELS compared with the Control, whereas both ELS and PSC decreased n-6 fatty acids ($P < 0.05$). Total PUFA and total UFA remained unchanged compared with the Control, although total LCFA increased when feeding ELS, probably due to LCFA increase in ELS diets.

The current findings indicate that soya bean meal can be completely substituted with PSC, when feeding dairy goats, without any decrease in milk production or sharp changes in fatty acid profile. Compared with partial substitution of soya bean meal with ELS, PSC had lower total n-3 fatty acids but similar total UFA, PUFA and LA/ALA ratio. Partially

https://doi.org/10.1017/S175173111700060X

substituted soya bean meal with ELS in hay-based diets may enhance goat milk with n-3 fatty acids, especially ALA and EPA, and lowering LA/ALA ratio. Higher proportions of OBCFA were also observed with feeding ELS but not with PSC, which might indicate different activities of rumen microbes and is worth to further study.

Acknowledgements

The authors thank the farmers for care of animals, as well as Birte Fischer-Kassebart for assistance in performing fatty acid analyses.

References

Allen MS 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83, 1598–1624.

Bernard L, Rouel J, Leroux C, Ferlay A and Faulconnier Y 2005. Mammary lipid metabolism and milk fatty acid secretion in Alpine goats fed vegetable lipids. Journal of Dairy Science 88, 1478–1489.

Bodas R, Manso T, Mantecon AR, Juarez M, de la Fuente AM and Gomez-Cortes P 2010. Comparison of the fatty acid profiles in cheeses from ewes fed diets supplemented with different plant oils. Journal of Agricultural and Food Chemistry 58, 10493–10502.

Chilliard Y and Ferlay A 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reproduction, Nutrition, Development 44, 467–492.

Chilliard Y, Ferlay A, Rouel J and Lamberet G 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. Journal of Dairy Science 86, 1751–1770.

International Committee for Animal Recording 2012. International agreement of recording practices. ICAR, Rome, Italy.

International Organization for Standardization 2002a. Milk fat – preparation of fatty acid methyl esters (International Standard ISO 15884|IDF 182:2002. The International Organization for Standardization and International Dairy Federation, Geneva, Switzerland.

International Organization for Standardization 2002b. Milk fat – determination of the fatty acid composition by gas-liquid chromatography (International Standard ISO 15885|IDF 184:2002. The International Organization for Standardization and International Dairy Federation, Geneva, Switzerland.

International Organization for Standardization 2010. Milk – determination of fat content – gravimetric method (International standard ISO 1211:2010(E) IDF 1:2010(E). The International Organization for Standardization and International Dairy Federation, Geneva, Switzerland.

Ivan M, Mir PS, Koenig KM, Rode LM, Neill L and Mir Z 2001. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Ruminant Research 41, 215–227.

Månsson HL 2008. Fatty acids in bovine milk fat. Food and Nutrition Research 52, 1–3, 10.3402/fnr.v52i0.1821.

Martínez-Marín AL, Gomez-Cortes P, Gomez-Castro AG, Juarez M, Perez-Alba LM, Perez Hernandez M and de la Fuente MA 2011. Animal performance and milk fatty acid profile of dairy goats fed diets with unsaturated plant oils. Journal of Dairy Science 94, 5359–5368.

Martínez-Marín AL, Gomez-Cortes P, Gomez-Castro G, Juarez M, Perez-Alba L, Perez Hernandez M and de la Fuente MA 2012. Effects of feeding increasing dietary levels of high oleic or regular sunflower or linseed oil on fatty acid profile of goat milk. Journal of Dairy Science 95, 1942–1955.

Mele M, Buccioni A, Serra A, Antongiovanni M and Secchiari P 2008. Lipids of goat's milk: origin, composition and main sources of variation. In Dairy goats feeding and nutrition (ed. A Cannas and G Pulina), pp. 47–65. CAB International, Wallingford, UK.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. The Journal of Agricultural Science 93, 217–222.

Mika A, Stepnowski P, Kaska L, Proczko M, Wisniewski P, Sledzinski M and Sledzinski T 2016. A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight. Obesity 24, 1669–1676.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ and Willett WC 2006. *Trans* fatty acids and cardiovascular disease. New England Journal of Medicine 354, 1601–1613.

Murković M, Hillebrand A, Winkler A, Leitner E and Pfannhauser W 1996. Variability of fatty acid content in pumpkin seeds (*Cucurbita pepo* L.). Zeitschrift für Lebensmittel Untersuchung und Forschung 203, 216–219.

National Research Council 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. The National Academies Press, Washington, DC, USA.

Nudda A, Battacone G, Usai MG, Fancellu S and Pulina G 2006. Supplementation with extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in goat milk. Journal of Dairy Science 89, 277–282.

Nudda A, Palmquist DL, Battaconea G, Fancellua S, Rassua SPG and Pulina G 2008. Relationships between the contents of vaccenic acid, CLA and n-3 fatty acids of goat milk and the muscle of their suckling kids. Livestock Science 118, 195–203.

Offor IF, Ehiri RC and Njoku CN 2014. Proximate nutritional analysis and heavy metal composition of dried *Moringa oleifera* leaves from Oshiri, Onicha L.G.A, Ebonyi State, Nigeria. Journal of Environmental Science, Toxicology and Food Technology 8, 57–62.

Onwuka GI 2005. Food analysis and instrumentation: theory and practice. Naphthali Prints, Lagos, Nigeria.

Pearson DA 1976. The chemical analysis of foods, 7th edition. pp. 6–25. Churchill Livingstone, Edinburgh, Scotland.

Queiroga RCRE, Melo Santos B, Pereira Gomes AM, João Monteiro M, Teixeira SM, Souza EL, Dias Pereira CJ and Estevez Pintado MM 2013. Nutritional, textural and sensory properties of Coalho cheese made of goats', cows' milk and their mixture. Food Science and Technology 50, 538–544.

Renna M, Lussiana C, D'Agostino M, Mimosi A and Fortina R 2013. Extruded linseed supplementation in dairy goat diet: effects on productive performance and fatty acid profile of bulk milk, fresh and ripened cheese. Journal of Animal and Veterinary Advances 12, 1550–1564.

Salter AM 2013. Dietary fatty acids and cardiovascular disease. Animal 7 (suppl. 1), 163–171.

Shingfield KJ, Bernard L, Leroux C and Chilliard Y 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4, 1140–1166.

Simopoulos AP 2011. Evolutionary aspects of diet: the omega-6/omega-3 ration and the brain. Molecular Neurobiology 44, 203–215.

Siri-Tarino PW, Sun Q, Hu FB and Krauss RM 2010. Saturated fat, carbohydrate, and cardiovascular disease. The American Journal of Clinical Nutrition 91, 502–509.

Vannice G and Rasmussen H 2014. Position of the Academy of Nutrition and Dietetics: dietary fatty acids for healthy adults. Journal of the Academy of Nutrition and Dietetics 114, 136–153.

Vasta V, Nudda A, Cannas A, Lanza M and Priolo A 2008. Alternative feed resources and their effects on the quality of meat and milk from small ruminants. Animal Feed Science and Technology 147, 223–246.

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM and Dewhurst RJ 2006. Factors affecting odd- and branched-chain fatty acids in milk: a review. Animal Feed Science and Technology 131, 389–417.

Zdunczyk Z, Minakowski D, Frejnagel S and Flis M 1999. Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. Nahrung 43, 392–395.

Zhang S, Wang Z and Xu S 2007. Optimization of the aqueous enzymatic extraction of rapeseed oil and protein hydrolysates. Journal of American Oil Chemists' Society 84, 97–105.