Feeding wheat dried distillers' grains with solubles increases conjugated linoleic acid and unsaturated lipids in ovine milk without adversely affecting milk yield

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SUPPLEMENTARY FILE

Materials & methods

Animals, management and experimental diets

The experiment was performed in the research farm of Agricultural Research Institute (**ARI**, Athalassa, Nicosia) in Cyprus. Animals were housed indoors, and all experimental procedures were carried out according to international guidelines Directive 2010/63/EU and approved by the corresponding departmental committee of the Cyprus University of Technology. Feed ingredients and the feed chemical analysis including FA composition are shown in Table S1 and S2, respectively. Dry matter intake (DMI) was monitored and feeds were offered manually, as a total mixed ration, two times per day after morning and evening milking. In each pen the animals were group fed the diets to 1.1 times their maintenance energy (0.401 MJ/kg of weight0.73) and milk production requirements [dietary ME (MJ/kg of milk): Y = (1.94 + 0.43X)/0.62, where X is the fat percentage and 0.62 the efficiency of utilization of dietary ME for milk production of Chios ewes; Economides, 1986]. Feed samples were collected at the beginning, at the middle and at the end of the trial, mixed per treatment and analyzed in triplicate using methods described previously (Symeou *et al.*, 2019). Intake measurements presented were taken during the first week of the experiment and after the 10 days of the adaptation period.

Measurements, sampling and analysis

All animals were machine milked (Fullwood, Shropshire, UK) twice daily (at 0430 h and 1630 h) and milk yields were recorded electronically (AfiMilk model Afifree 155, SAE Afikim Kibbutz, Israel). Raw milk samples for the determination of the lipid profile were collected from each ewe during two consecutive milkings at the end of each sampling week (days 17, 24, 31, 38 and 45 of the experiment), mixed (morning and evening) and immediately transferred with the use of a cool box (4 ^{o}C) to the laboratory for chemical composition determination by the use of combined thermo-optical procedures (Lactostar 3510, Funke Gerber, Berlin, Germany). Milk subsamples were stored at -80°C for further analyses for FA composition. 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 x 0.25 mm x 0.2 μm column (Agilent CP-Sil 88 fused silica capillary column, Agilent, Santa Clara, United States) with a 1:20 split ratio. The column was held for 4 min at 70°C after injection, increased at 13°C/min to 175°C, and then held at that temperature for a further 27 min. The temperature was then raised to 215°C at 4°C/min at which it was held for a further 36 min. Helium was the carrier gas at 1 mL/min, with both injector and interface temperatures of 225°C. Chromatographic profiles were analysed 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 authentic standards (Supelco 37-FAME standard mix, CLA *cis*-9, *trans*-11, CLA *trans*-10, *cis*-12, C18:1 *trans*-11; Sigma-Aldrich, Gillingham, UK) and using the National Institute of Standards and Technology 08 and 21 mass spectral libraries and cross referencing with chromatograms-spectrograms reported in the literature (Kramer *et al.*, 2008; Tsiafoulis *et al.*, 2014). All FAME peaks identified were quantitated by peak integration and individual FAME expressed as a percentage of the total fat.

Milk atherogenic index (**AI**) was determined using the formula proposed by Ulbricht and Southgate, (1991) AI= (C12:0 + 4 x C14:0 + C16:0)/ (ΣMUFA + ΣPUFA) and the desaturation index (**DI**) was determined using the formula suggested by Garnsworthy *et al.*, (2010): DI = (C14:1 *cis*-9 x 100)/ (C14:0 + C14:1 *cis*-9). Fat-corrected milk yield at 6% of fat content (FCM 6%) was estimated according to Mavrogenis and

Papachristoforou (1988) for Chios sheep: FCM 6% = milk yield x (0.453 + 0.0912 x fat%).

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Table S1. Ingredients of diets contained 0 (control, DG0), 6 (DG6) and 12 (DG12) g of wheat - based dried distillers' grains with solubles per 100g DM

Item, % DM	Diet		
	DG0	DG6	DG12
Barley hay	40	40	40
Barley grain	15.6	15.6	15.6
Corn grain	18.12	15.9	13.38
Wheat bran	4.8	4.8	4.8
Sugar beet pulp	6	6	6
Soya bean meal[1]	8.4	4.68	1.2
Sunflower meal[2]	4.8	4.8	4.8
Wheat DDGS	-	6	12
Mineral and vitamin mix[3]	2.28	2.22	2.22

[1]Containing 47% CP, [2]Containing 35% CP, [3]Containing (% DM): Magnesium oxide (5.26), Sodium Biocarbonate (21.05), Limestone (57.9), Sodium chloride (5.26), Monocalcium phosphate (5.26%), Micro - mineral and vitamin premix (5.26)

Table S2. Chemical composition of the DDGS feed as well as the chemical composition and the fatty acid profile of the experimental diets contained 0 (control, DG0), 6 (DG6) or 12 (DG12) g of wheat - based dried distillers' grains with solubles (DDGS) per 100g DM.

Item	DDGS	Diet			SEM	P-value[1]
		DG0	DG6	DG12		
Chemical composition						
Dry matter, % as fed	90.1	89.60	89.50	90.20	0.11	NS
Crude protein, % DM	26.41	14.45	14.61	14.32	0.12	NS
Crude fiber, % DM	6.30	16.83	16.75	17.17	0.13	NS
Ether extract, % DM	6.31	1.28^{c}	1.42^{b}	1.82^{a}	0.02	***
Ash, % DM	4.23	7.38	7.22	7.37	0.05	NS
aNDF, % DM	31.68	33.37^{c}	34.09^{b}	35.07^{a}	0.40	**
ADF, % DM	16.11	20.64^{c}	21.33^{b}	22.76^{a}	0.48	***
ME (MJ/kg)[2]	10.98	11.79	11.83	11.78	0.03	NS
Fatty acid profile, % of total fatty acid						
C16:0		16.84^{a}	17.03^{b}	18.39^{a}	0.52	*
C18:0		4.12	3.52	2.79	0.46	NS
C18:1 *cis*-9		29.11	29.43	28.85	0.37	NS
C18:2n-6		44.44	44.74	45.00	0.31	NS
C18:3n-3		3.89	3.86	3.69	0.08	NS

[a-b]Means within a row not sharing a common superscript differ, [1]Probability of significant effects; *P < 0.05; **P < 0.01; ***P < 0.001; NS = Non significance, [2]Calculated from NRC (2001)