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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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Research Article

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Abstract

The aim of this research communication was to examine the effect of dietary supplementation with wheat-based dried distillers' grains with solubles (DDGS), a by-product of bioethanol production, on yield, composition, and fatty acid (FA) profile of ewe milk. Forty-five purebred mid-lactating Chios ewes (average milk yield 2.23 kg/d in 96 ± 5 d in lactation) were offered three iso-nitrogenous and iso-energetic diets (15 animals per diet) for a 10 d adaptation period followed by a 5-week recording and sampling period. The diets contained 0, 6, and 12% DDGS on DM basis for the DG0, DG6, and DG12 treatment, respectively, as a replacement of concentrate mix, whilst concentrate-to-forage ratio remained at 60:40 in all treatments. Individual milk yield, milk composition, and FA profile were recorded weekly and analyzed using a complete randomized design with repeated measurements. No significant differences were observed among groups concerning dry matter intake (overall mean of 2.59 kg/d), milk yield or 6% fat-corrected milk and milk protein percentage or protein yield. Milk fat percentage was decreased in the DG12 (4.76%) compared to DG0 (5.69%) without, however, significantly affecting the daily output of milk fat. The concentration of all major saturated FA between C4:0 to C16:0 was reduced, whereas long-chain (>16 carbons), mono-unsaturated and poly-unsaturated FAs were increased in the milk of DDGS groups. Among individual FA, increments of oleic acid and C18:1 *trans*-monoenes like C18:1 *trans*-10 and C18:1 *trans*-11 were demonstrated in DG12 group, whereas linoleic and conjugated linoleic acid (CLA *cis*-9, *trans*-11) were elevated in both DDGS groups compared to control. Changes in FA profile resulted in a decline in the atherogenic index of milk by 20% and 35% in DG6 and DG12 treatments, respectively, compared with control. In conclusion, feeding DDGS to dairy ewes increased the levels of unsaturated FA that are potentially beneficial for human health without adversely affecting milk, protein or fat yield.

The rapid growth of bioethanol production from grains such as corn, wheat and sugarcane has generated significant quantities of by-products that can be used as alternative feedstuffs in dairy animals (Schingoethe *et al.*, 2009). There are two different bioethanol production processes, wet milling and dry grind, with the latter predominating (Schingoethe *et al.*, 2009). During the dry grinding process, extractions may exist in different forms including dried distillers' grain (DDG) and dried distillers grain with solubles (DDGS). DDGS represents an interesting supplement for ruminants due to its high protein, fat, and energy content (30%, 11.2% and 9.4 MJ NE_l/kg in DM, respectively, Schingoethe *et al.* 2009). High concentration of DDGS in the diets may cause milk fat depression when it coincides with reduced forage NDF content as shown in studies in cows with increasing concentration of DDGS in diet (Cyriac, 2005; Leonardi *et al.*, 2005). In most studies, this reduction in fat percentage was not accompanied by a reduction in milk or fat yield although a concomitant reduction in milk yield with 25% DDGS inclusion level has been reported (Testroet *et al.*, 2015). Nevertheless, the majority of studies in dairy cattle investigating DDGS from different grains with inclusion rates up to around 20% of DM intake showed that milk production and fat yield were increased or not adversely affected, when diets contained adequate levels of forage fiber (Schingoethe *et al.*, 2009; Chibisa *et al.*, 2012; Gaillard *et al.*, 2017a, 2017b). Furthermore, the use of DDGS in cows enriched milk lipids with fatty acids (**FA**) potentially beneficial for human health; the content of saturated fatty acid (**SFA**) was decreased and the levels of long-chain FA, mono-unsaturated FA (**MUFA**) and poly-unsaturated FA (**PUFA**) including conjugated linoleic acid (**CLA** *cis*-9, *trans*-11) were increased (Leonardi *et al.*, 2005; Anderson *et al.*, 2006; Sasikala-Appukuttan *et al.*, 2008; Abdelqader *et al.*, 2009; Kurokawa *et al.*, 2013; Testroet *et al.*, 2015; Gaillard *et al.*, 2017a, 2017b).

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Table 1. Milk production and chemical composition of milk from ewes fed diets contained 0 (control, DG0), 6 (DG6) or 12 (DG12) g of wheat – based dried distillers' grains with solubles per 100 g DM

	Treatment				*P*-value[1]		
Item	DG0	DG6	DG12	SEM	*D*	*T*	*D* × *T*
DMI, kg/d	2.62	2.59	2.55	0.02	NS	–	–
Yield, kg/d							
Milk	1.63	1.62	1.75	0.06	NS	***	NS
6% FCM[2]	1.55	1.56	1.53	0.06	NS	***	NS
Fat	0.098	0.097	0.088	0.003	NS	***	NS
Protein	0.081	0.080	0.084	0.002	NS	***	†
Milk composition, %							
Fat	5.69[a]	5.60[a]	4.76[b]	0.18	**	***	NS
Protein	4.80	4.73	4.70	0.03	NS	**	*
Lactose	4.94	4.83	4.85	0.03	NS	NS	NS
SNF[3]	10.88	10.64	10.72	0.07	†	†	***

[a–c]Means within a row not sharing a common superscript differ due to the different diet examined ($P < 0.05$), [1]Probability of significant effects due to diet (*D*), time (*T*), and their interaction (*D* × *T*); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, Non-significant; †$P < 0.1$: tendency, [2]6% FCM, milk yield × (0.453 + 0.0912 × fat), [3]SNF, Solids non-fat.

With regards to small ruminants, only two studies have investigated the use of DDGS in lactating animals (Cais-Sokolińska *et al*., 2015; Alshdaifat and Obeidat, 2019). In the latter the inclusion of 20 and 30% DDGS in the diet (DM basis) of dairy Awassi ewes increased milk yield without affecting the percentages of milk fat or protein, while the FA content of milk fat was not investigated. The former study (Cais-Sokolińska *et al*., 2015) examined only the chemical composition of sheep and goat milk and their products, and not milk yield. They reported that the inclusion of maize DDGS at the level of 3.5% (on DM basis) did not affect milk fat and protein percentages, but did affect FA content showing increased concentrations of total PUFA and *trans* – MUFA for both ovine and caprine milk, and reduced SFA, as well as increased MUFA and CLA content in caprine but not in ovine milk.

The present study was designed to investigate the effects of feeding DDGS on milk production and content, as well as on milk FA composition of dairy Chios ewes during a five-week sampling period in mid lactation. The hypothesis was that inclusion of DDGS at rates of 6% and 12% in dietary DM with wheat DDGS would positively affect yield, composition or FA profile of milk, suggesting therefore its use as an alternative protein-rich supplement in sheep rations.

Materials and methods

Forty–five multiparous purebred, mid-lactating Chios ewes (average ± SD: 96 ± 5 d in lactation) were randomly distributed to 9 pens of 5 animals and allocated at random to 3 experimental feeding treatments (3 pens or 15 animals per treatment) resulting in overall means (± SEM) of 2.23 ± 0.01 kg for daily milk yield and 59.3 ± 0.4 kg for live weight. Feeding treatments contained 0, 6, and 12% on DM of wheat DDGS replacing corn grain and soybean meal (DG0, DG6 and DG12 treatments, respectively online Supplementary Table S1).

As shown in this table, animals received also barley hay of good quality as forage, obtaining a concentrate to forage ratio of 60:40 for all treatments. Feed ingredients and chemical composition are shown in Tables S1 and S2, respectively, in the online supplementary material. The three feeding regimes were isoenergetic and iso-nitrogenous and were offered for a 10 d adaptation period followed by a 5-week recording and sampling period. All animals were machine milked twice daily, and milk yields were recorded electronically. Milk samples for the determination of milk composition and lipid profile were collected weekly from each ewe for five consecutive weeks. Measurements for total fat, protein, and solids non-fat were performed with the use of Lactostar 3510 (Funke Gerber, Berlin, Germany) according to Tzamaloukas *et al.* (2015). Lipids from milk and feeds were extracted and methylated as described by Symeou *et al.* (2019) and (2020), and Tsiafoulis et al. (2014), respectively. Milk atherogenic (**AI**) and desaturation index (**DI**) were determined and fat-corrected milk yield at 6% of fat content was estimated. Further details of all methodologies and calculations are given in the online Supplementary Materials and Methods.

Statistical analysis

Performance, milk content and milk FA composition data were analyzed using repeated measures analysis for a Completely Randomized Design, using SAS PROC MIXED (SAS version 9.4. SAS Institute Inc., Cary, NC). The model included the fixed effects of diet (*D*), time (*T*), and their interaction ($T \times D$) and the random effect of ewes and pen. Statistical significance declared at $P < 0.05$. *P*-values between >0.05 and <0.10 were interpreted as trends toward significance. Data referring to DMI, chemical and FA composition of feeds were analyzed using one-way ANOVA with 3 replications, respectively.

Results and discussion

Table 1 shows the DM intake, 6% fat-corrected milk, the daily yield of milk, fat, and protein, as well as the composition of milk. DDGS inclusion in the ewe diets did not affect intake, the

Table 2. Fatty acid composition (expressed as a percentage of total fatty acid methyl esters) of milk from ewes fed diets contained 0 (control, DG0), 6 (DG6) or 12 (DG12) g of wheat – based dried distillers' grains with solubles per 100 g DM

	Treatment				*P*-value[1]		
Item	DG0	DG6	DG12	SEM[3]	*D*	*T*	*D* × *T*
C4:0	2.73[a]	2.79[a]	2.67[b]	0.05	*	***	†
C6:0	2.69[a]	2.65[a]	2.33[b]	0.08	***	***	NS
C7:0	0.08	0.07	0.08	0.009	NS	†	†
C8:0	3.11[a]	3.01[a]	2.55[b]	0.08	**	***	NS
C10:0	7.95[a]	7.23[b]	6.17[c]	0.18	**	***	NS
C10:1 *cis*-9	0.46[a]	0.40[b]	0.34[c]	0.01	***	**	NS
C12:0	5.84[a]	5.04[b]	4.46[c]	0.13	***	NS	NS
C12:1 *cis*-9	0.10[a]	0.08[b]	0.07[b]	0.005	NS	**	**
C13:0	0.33	0.25	0.30	0.02	†	NS	*
C14:0	12.28[a]	11.35[b]	10.36[c]	0.17	***	**	NS
C14:0 *iso*	0.10	0.10	0.11	0.01	NS	NS	NS
C14:1, *cis*-9	0.30[a]	0.21[b]	0.20[b]	0.01	NS	***	**
C15:0	1.72[a]	1.46[b]	1.75[a]	0.06	***	***	***
C15:0 *iso*	0.25[a]	0.25[ab]	0.23[b]	0.008	NS	***	NS
C15:0 *antiso*	0.75[a]	0.67[b]	0.69[b]	0.02	NS	NS	NS
C16:0	22.26[a]	21.86[a]	20.61[b]	0.34	*	**	NS
C16:0 *iso*	0.30	0.31	0.31	0.01	NS	NS	NS
C16:1 *cis*-9	2.17	2.02	2.00	0.09	NS	NS	NS
C16:1 *trans*-9	0.60	0.57	0.62	0.02	NS	NS	NS
C17:0	0.74[ab]	0.73[b]	0.80[a]	0.02	*	***	NS
C17:0 *iso*	0.46	0.45	0.44	0.02	NS	NS	NS
C17:0 *antiso*	0.71	0.67	0.70	0.04	NS	NS	NS
C17:1 *cis*-9	0.29[b]	0.29[b]	0.34[a]	0.01	*	NS	NS
C18:0	5.22	6.20	5.39	0.19	†	†	NS
C18:1 *trans*-9	0.35	0.39	0.48	0.06	†	NS	NS
C18:1 *trans*-10	0.46[b]	0.53[b]	0.77[a]	0.05	**	**	*
C18:1 *trans*-11	3.10[b]	3.26[b]	4.99[a]	0.32	**	*	NS
C18:1 *trans*-16	0.10	0.09	0.12	0.02	NS	NS	NS
C18:1 *cis*-9	14.03[b]	15.24[b]	15.43[a]	0.27	*	***	NS
Other *cis*-C18:1[2]	1.07	1.18	1.32	0.06	NS	***	NS
C18:2 *trans*-9, *cis*-13/ *trans*-8, *cis*-12	0.19	0.23	0.26	0.007	NS	**	†
C18:2 *trans*-8, *cis*-13	0.08[b]	0.11[a]	0.12[a]	0.006	*	NS	**
C18:2 *trans*-11, *cis*-15	0.13[b]	0.18[a]	0.19[a]	0.02	*	NS	NS
C18:2 *trans*-9, *cis*-12	0.04	0.04	0.06	0.01	NS	NS	NS
C18:2n-6	3.65[c]	4.15[b]	5.21[a]	0.14	***	NS	†
C18:3n-6	0.10	0.08	0.09	0.005	†	**	NS
C18:3n-3	0.33	0.33	0.41	0.01	NS	***	NS
CLA *cis*-9, *trans*-11	0.55[b]	0.67[a]	0.72[a]	0.03	*	***	**
CLA *trans*-9, *cis*-11	0.08[b]	0.08[b]	0.15[a]	0.01	**	NS	NS
CLA *trans*-10, *cis*-12	0.05	0.05	0.06	0.004	NS	***	**
C20:4n-6	0.23	0.23	0.26	0.01	NS	***	NS

(*Continued*)

Table 2. (*Continued.*)

Item	Treatment			SEM[3]	*P*-value[1]		
	DG0	DG6	DG12		*D*	*T*	*D* × *T*
SCFA[3]	8.61[a]	8.52[a]	7.63[b]	0.16	***	***	*
MCFA[4]	54.43[a]	50.69[b]	47.23[c]	0.53	***	***	NS
LCFA[5]	34.97[c]	39.01[b]	43.05[a]	0.62	***	NS	NS
<C16	36.87[a]	33.51[b]	30.90[c]	0.53	***	***	NS
>C16	34.97[c]	39.01[b]	43.05[a]	0.62	***	NS	NS
SFA	68.70[a]	66.62[b]	61.29[c]	0.71	***	***	NS
MUFA	23.69[b]	25.41[b]	28.12[a]	0.41	***	***	NS
PUFA	5.66[c]	6.35[b]	7.80[a]	0.21	***	***	NS
AI[6]	2.82[a]	2.27[b]	1.82[c]	0.06	***	NS	*
DI[7]	2.84	2.41	2.50	0.11	NS	***	NS

[a–c]Means within a row not sharing a common superscript differ due to the different diet examined ($P < 0.05$), [1]Probability of significant effects due to diet (D), time (T), and their interaction (D × T); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, NS, Non-significant; †$P < 0.1$: tendency, [2]Other C18:1 *cis* = C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:1 *cis*-14; C18:1 *cis*-15, [3]SCFA = short-chain fatty acids (C4:0 to C8:0), [4]MCFA = medium-chain fatty acids (C10:0 to C16:1), [5]LCFA = long-chain fatty acids (C17:0 and above), [6]Atherogenic index = (C12:0 + 4 × C14:0 + C16:0)/(ΣMUFA + ΣPUFA), [7]Desaturation index = (C14:1 *cis*-9/C14:0 + C14:1 *cis*-9) × 100, Unidentified picks were less than 2% in all groups.

yield of milk, or the fat-corrected milk. There is only one study (Alshdaifat and Obeidat, 2019) testing corn DDGS in dairy sheep production. This reported increased milk yields with DDGS inclusion in the diets (daily milk production of 0.95, 1.17 and 1.19 kg/d for inclusion rates of 0, 20 and 30% DM, respectively). Small increases (not always significant) have also been reported in some studies with lactating cows (Janicek *et al.*, 2008; Chibisa *et al.*, 2012; Benchaar *et al.*, 2013) and it has been attributed to either the slightly increased energy density, due to higher fat content in DDGS diets, or to greater DM intake and, therefore, higher energy acquisition by the animals offered DDGS. Nevertheless, most cow studies with isoenergetic and isonitrogenous experimental diets, and similar intakes, as those applied in the present experiment, reported no effect on milk yield, when the applied inclusion level of DDGS was up to 30% DM (Abdelqader *et al.*, 2009; Gaillard *et al.*, 2017a, 2017b; Testroet *et al.*, 2018)

DDGS inclusion did not affect milk protein levels in the present study (Table 1), a fact that was also reported in a previous related study in sheep (Alshdaifat and Obeidat, 2019). Nonetheless, the results in previous studies with cows on the effect of DDGS on milk protein content are controversial, reporting either an increase (Testroet *et al.*, 2015), or a decrease (Kleinschmit *et al.*, 2006; Benchaar *et al.*, 2013; Kurokawa *et al.*, 2013), or no significant effect (Anderson *et al.*, 2006; Janicek *et al.*, 2008; Sasikala-Appukuttan *et al.*, 2008; Chibisa *et al.*, 2012). Reviewing the studies in cows, Kalscheur *et al.* (2012) concluded that milk protein content is rarely reduced by feeding DDGS, unless protein is limited in the diet or DDGS levels are higher than 30% DM of the diet reflecting the high rumen undegradable protein of the diet, which may result in a reduction of provided lysine for milk protein synthesis in the mammary gland. However, none of these prerequisites took place in our study where adequate feed protein levels and lower DDGS inclusion rates were used.

Milk fat percentage in the present study was reduced with the inclusion rate of 12%, but not with 6% of DDGS in the diet (on DM basis) compared to control, without however affecting the fat yield (Table 1). Reduction of milk fat was not observed in a previous study in sheep with either 20 or 30% DM inclusion rates of corn DDGS (Alshdaifat and Obeidat, 2019). Nevertheless, it is well established in lactating cows that the addition of high levels of DDGS in diets could result in a decrease of milk fat percentages when NDF content and forage participation in the diets are low, a fact that has also been observed in practice (Schingoethe *et al.*, 2009). A meta-analysis by Kalscheur *et al.* (2012) demonstrated that milk fat depression in cows fed with DDGS occurred only when diets contained less than 22% forage NDF and 50% total forage. This finding led these authors to suggest that the key of maintaining milk fat percentage is to provide not only adequate NDF and specific percentage of forages in the diet but also sufficient amounts of effective fiber (e.g. hay as opposed to corn silage). Although our study was designed to provide adequate dietary NDF content (34 and 35% DM in DG6 and DG12, respectively) and the barley straw component was at 40% of the diet DM (usual practice in Cyprus), it is not known if these practices were adequate to prevent milk fat reduction in sheep with the 12% DDGS inclusion in the diet.

All individual FA and their aggregated groups determined in milk of DG0, DG6, and DG12 ewes are presented in Table 2. The DDGS inclusion had a marked effect on milk FA composition by reducing SFA and increasing unsaturated FAs in both DG6 and DG12 treatments. The content of saturated FAs with less than 16 carbons were linearly reduced with increased DDGS proportion in the ewe diets, while the only saturated FA that came close to increase was C18:0 and this difference was not significant. These results are in line with those of a previous study in ewes (Cais-Sokolińska *et al.*, 2015) and with many studies conducted in cows (Leonardi *et al.*, 2005; Anderson *et al.*, 2006; Sasikala-Appukuttan *et al.*, 2008; Abdelqader *et al.*, 2009; Hippen *et al.*, 2010; Kurokawa *et al.*, 2013; Gaillard *et al.*, 2017a, 2017b) showing diminished de novo FA percentages and increased levels of long-chain FA (LCFA) as a result of DDGS dietary inclusion. The reduction of SFA in the present study was accompanied by a linear increase in total PUFA and MUFA with the increasing concentrations of DDGS in the

sheep diets. Individual FA with a special interest for human health such as oleic, vaccenic, linoleic, and conjugated linoleic acid (CLA *cis*-9, *trans*-11), were linearly elevated with the addition of 6% and 12% DDGS in diet DM (Table 2). Similar effects have been reported in studies that examined diets from 5 up to 20% DM inclusion levels of DDGS in cows (Kalscheur *et al.*, 2012; Kurokawa *et al.*, 2013; Gaillard *et al.*, 2017a, 2017b) and also observed in ewes with low DDGS inclusion (3.5% in diet DM, Cais-Sokolińska *et al.* 2015).

The reduction of FA with carbon chain less than 16 is due to either a higher secretion of LCFA from the blood and/or a lower de novo synthesis of FAs in the mammary gland (Chilliard *et al.*, 2007; Dorea and Armentano, 2017). DDGS diets contained higher levels of ether extract than the control diet, indicating that ewes fed with DDGS consumed greater amounts of LCFA, including PUFA such as linoleic acid. Furthermore, *de novo* FA secretion can be inhibited by bioactive PUFA, produced during incomplete ruminal biohydrogenation of dietary LCFA, particularly CLA *trans*-10, *cis*-12 and *trans* monoenes, such as C18:1 *trans*-10 (Dorea and Armentano, 2017) with the latter found elevated in the milk of ewes fed DDGS in our study (Table 2). With regards to the predominant CLA isomer, *cis*-9, *trans*-11, (rumenic acid), it is well known that the majority of rumenic acid in the milk fat is synthesized endogenously, in the mammary gland through the action of mammary Δ9-desaturase with the substrate being vaccenic acid, while a small amount of this specific CLA originates from ruminal biohydrogenation of unsaturated FA by rumen bacteria (Dorea and Armentano, 2017). Thus, the elevated proportions of rumenic acid observed in the milk of DDGS groups are either due to the higher concentrations of vaccenic acid observed in those groups (Table 2) or due to the incomplete biohydrogenation of longer FA that took place in the rumen of ewes fed with the DDGS diets.

In conclusion, the observed changes in the FA profile as a consequence of feeding dried distillers' grains with solubles resulted in an enrichment with beneficial FA and a reduction of atherogenic index of sheep milk by 20% (6% by DM inclusion) and 35% (12% inclusion), suggesting an improvement of health-related attributes of milk and possibly related dairy products. These results are useful for exploiting a cost-effective bioethanol byproduct to improve the FA profile of ewe milk and possibly other related dairy products without adverse effects on milk production.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000443

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