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# The effect of dietary inclusion of olive tree leaves and grape marc on the content of conjugated linoleic acid and vaccenic acid in the milk of dairy sheep and goats

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Olive tree leaves (OTL) and grape marc (GM) are by-products with high linolenic (LNA) and linoleic (LA) acid content, respectively, which can be used as dietary ingredients to increase the *cis*-9 *trans*-11 conjugated linoleic acid (CLA) content of milk fat in sheep and goats. An experiment was conducted with 16 Friesian ewes and 16 Alpine goats to study the effect of OTL and GM inclusion in sheep and goat diets on their milk fatty acid profile, with emphasis on *cis*-9, *trans*-11 CLA and vaccenic acid (VA). Ewes and the goats were fed the control (C) diets from parturition to the 90 days in milk (DIM) and then both groups were divided into two sub-groups (treatments). The control groups of both species continued to be fed the C diets, whereas the treated groups were gradually switched over a 2-week period (DIM=91–105) from the C diets to that of treatment 1, which contained air-dried OTL. These OTL diets were fed ad libitum for 1 month (DIM=106–135). After that period, the same treated groups, after 2 weeks of gradual adaptation (DIM=136–150), were switched to treatment 2, which contained air-dried GM. The GM diets were fed ad libitum for 1 month (151–180 DIM). Concentrations of polyunsaturated fatty acids (PUFA) and mono-unsaturated fatty acids increased significantly in milk fat of sheep fed OTL *v*. C. For goats, only the PUFA in milk fat was increased by feeding OTL compared with C. Relative to C, GM increased significantly the concentration of PUFA only in milk fat of sheep. OTL and GM diets increased the *cis*-9, *trans*-11 CLA and VA content in milk fat, compared with C, only in sheep. GM caused a sharp increase in 18:0 only in sheep milk fat, while the OTL diet increased significantly the 18:0 in milk fat of goats. GM and OTL diets also had opposite effects on the 18:1/18:0 ratio of sheep milk fat. In conclusion, OTL and GM, when included in sheep diets altered the milk fatty acid profile with a pronounced increase in *cis*-9, *trans*-11 CLA and VA contents. The results show that the response of sheep and goats to OTL and GM diets was different, suggesting a species difference that needs further investigation.

**Keywords:** Milk fatty acids, dietary manipulation.

Conjugated linoleic acid (CLA) represents a mixture of positional and geometric isomers of C18 fatty acid with two double bonds. The CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have been shown to have a range of positive health effects that include anti-carcinogenic, anti-atherogenic, anti-obesity, anti-diabetic and immune system enhancement (McGuire & McGuire, 2000; Larsson et al. 2005). Ruminant dairy products are the major dietary sources of CLA, and its isomer *cis*-9, *trans*-11 is approximately 78–89% of the total CLA in ovine milk fat (Antongiovanni et al. 2004).

The *cis*-9, *trans*-11 CLA in milk fat is produced primarily in the mammary gland by $\Delta^9$-desaturase from vaccenic acid (*trans*-11 18:1, VA), an intermediate formed in the rumen by biohydrogenation of linolenic (*cis*-9, *cis*-12, *cis*-15 18:3, LNA) and linoleic (*cis*-9, *cis*-12 18:2, LA) acids (Bauman et al. 2003). This *cis*-9, *trans*-11 CLA is also an intermediate in rumen biohydrogenation of LA, and a portion of it is absorbed to provide the remainder of the *cis*-9, *trans*-11 CLA in milk fat (Bauman et al. 2000).

Studies on milk fat CLA in sheep and goats have mainly focused on dietary sources of variation (Addis et al. 2006;

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**Table 1.** Chemical composition of dietary ingredients and the whole diet, and average daily intake of dietary ingredients in sheep and goats†

| Composition of diet, g/kg DM‡ | Control | Olive tree leaves | Grape marc |
|---|---|---|---|
| Sheep | | | |
| Alfalfa hay | 252 | — | 221 |
| Wheat straw | 252 | — | 221 |
| Concentrates | 496 | 249 | 362 |
| Olive tree leaves | — | 751 | — |
| Grape marc | — | — | 196 |
| Average DM intake, kg/d | 2·0 | 2·0 | 2·4 |
| Goats | | | |
| Alfalfa hay | 192 | — | 174 |
| Wheat straw | 142 | — | 131 |
| Concentrates | 666 | 298 | 506 |
| Olive tree leaves | — | 702 | — |
| Grape marc | — | — | 189 |
| Average DM intake, kg/d | 2·6 | 2·9 | 2·6 |
| Composition of consumed diet, g/kg DM | | | |
| Sheep | (*n*=6) | (*n*=6) | (*n*=6) |
| Organic matter | $932^{a}\pm4·2$ | $920^{b}\pm1·2$ | $913^{c}\pm7·5$ |
| Crude protein | $121\pm1·1$ | $118\pm2·4$ | $119\pm1·4$ |
| Ether extract | $27^{c}\pm1·1$ | $57^{a}\pm2·4$ | $36^{b}\pm1·7$ |
| Neutral dergent fibre | $400^{b}\pm6·4$ | $327^{c}\pm3·5$ | $443^{a}\pm5·4$ |
| Acid detergent fibre | $292\pm2·4$ | $219\pm3·4$ | $347\pm4·3$ |
| Goats | (*n*=6) | (*n*=6) | (*n*=6) |
| Organic matter | $940^{a}\pm1·0$ | $915^{c}\pm0·6$ | $924^{b}\pm0·8$ |
| Crude protein | $140^{a}\pm2·1$ | $121^{b}\pm1·6$ | $133^{a}\pm4·1$ |
| Ether extract | $30^{c}\pm1·2$ | $64^{a}\pm1·1$ | $38^{b}\pm0·8$ |
| Neutral detergent fibre | $305^{b}\pm5·8$ | $312^{b}\pm5·0$ | $352^{a}\pm7·3$ |
| Acid detergent fibre | $225^{b}\pm2·9$ | $211^{b}\pm4·4$ | $278^{a}\pm3·3$ |

† Means with different superscript (a, b, c) in each row for each parameter differ significantly ($P\leqslant0·05$)
‡ DM, dry matter

Nudda et al. 2006), seasonal variation (Nudda et al. 2005; Tsiplakou et al. 2006a) and physiological factors (Tsiplakou et al. 2006b). Diet is the main factor that influences milk fat contents of CLA and VA, the highest content of both fatty acids being found in milk of sheep and goats when their diet contains large amounts of LNA and LA (Chilliard et al. 2003; Nudda et al. 2006).

Olive tree leaves (OTL) and grape marc (GM) have traditionally been used in the Mediterranean area as alternative feeds for sheep and goats during periods of scarce feed supplies. OTL and GM are high in LNA and LA respectively, which can be used as substrate for *cis*-9, *trans*-11 CLA and VA biohydrogenation in the rumen. The studies reported with these by-products focused on their nutritive value (Fegeros, 1985; Zervas et al. 1993; Martín García et al. 2003) but no information is available on the effects of these by-products on milk fatty acid profile in sheep and goats.

Thus the objective of this study was to determine the effect of dietary inclusion of OTL and GM on milk FA profile in sheep and goats, with emphasis on *cis*-9, *trans*-11 CLA and VA.

## Materials and Methods

### *Animals and experimental design*

Sixteen 2–4-year-old Friesian dairy ewes weighing $65\pm2·3$ kg and sixteen 2–5-year-old Alpine dairy goats weighing $55\pm2·7$ kg were kept indoors in two groups under the same management throughout the experimental period. The body condition score (BCS) of ewes, assessed according to Russel et al. (1969), was $3·2\pm0·15$ and that of goats $2·7\pm0·16$ in a range from of 1 (very poor) to 5 (very fat). Lambing and kidding started in December and lasted 3 weeks. From parturition to the third month of lactation [days in milk (DIM) 1–90], sheep and goats were fed a diet based on alfalfa hay, wheat straw and concentrates (control diets, C). The composition and the average daily amount of diets consumed by sheep and goats are in Table 1. Three months post partum (DIM=91) the ewes and the goats were divided into two homogenous sub-groups (*n*=8) that were balanced by age, body weight and milk yield.

The control groups of both species continued to be fed the C diets, whereas the treated groups were gradually

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**Table 2.** Average (±SEM) daily milk yield and composition in sheep and goats throughout the experimental periods

| | DIM†=1–90 | DIM=106–135 | | DIM=151–180 | |
|---|---|---|---|---|---|
| | Control | Control | OTL‡ | Control | Grape marc |
| Sheep | | | | | |
| *n* | 16 | 8 | 8 | 8 | 8 |
| Milk yield, g/d | 1275±18 | 1206±13 | 1190±20 | 1171±16 | 1158±26 |
| Fat, g/kg | 68·9±1·3 | 69·8±1·7 | 69·4±1·8 | 70·1±2·0 | 72·9±3·3 |
| Protein, g/d | 54·8±0·5 | 56·3±0·6 | 57·1±0·7 | 57·0±0·8 | 53·2±1·4 |
| Goats | | | | | |
| *n* | 16 | 8 | 8 | 8 | 8 |
| Milk yield, g/d | 3100±55 | 2912±48 | 2850±89 | 2738±94 | 2702±100 |
| Fat, g/kg | 32·7±0·7 | 34·2±0·9 | 34·6±1·0 | 34·4±0·8 | 35·2±1·2 |
| Protein, g/d | 31·3±0·4 | 31·4±0·5 | 31·8±0·6 | 31·7±0·7 | 31·6±0·7 |

† Days in milk
‡ Olive tree leaves

switched over a 2-week period (DIM=91–105) from the C diets to that of treatment 1, containing air-dried OTL from olive cleaning. Thus, the OTL diets consisted of OTL and concentrates (Table 1) and were fed ad libitum for one month (DIM=106–135).

After that period, the same treated groups after 2 weeks of adaptation (DIM=136–150) were gradually switched from OTL to treatment 2 diet, which contained air-dried GM. GM diets consisted of alfalfa hay, wheat straw, GM and concentrates (Table 1) and were fed ad libitum for one month (DIM=151–180).

The average daily milk yield and composition in sheep and goats throughout the experimental period are shown in Table 2. All animals were fed twice a day at 8.00 and 17.00 ad libitum with daily feed intake recorded. Water was freely available at all times. C, OTL and GM diets were formulated according to animal species requirements (Zervas et al. 2004) by taking into account their maintenance (average body weight) and lactation (milk yield and milk composition, Table 2) requirements. The control groups of sheep and goats were fed a constant amount of 2·0 and 2·6 kg of the C diets, respectively, throughout the experimental period; there were no refusals.

OTL and GM were offered for ad-libitum consumption during the adaptation periods (DIM=91–105 for OTL and DIM=136–150 for GM) where the potential of their consumption was determined. Thus, OTL were consumed at 1·5 kg DM/d and 2·0 kg DM/d and GM at 0·47 kg DM/d and 0·49 kg DM/d by sheep and goats respectively. The offered amount of concentrates (Table 1) was calculated based on these OTL and GM intakes and on the respective requirements of the sheep and goats (Table 2). There were no refusals at each feeding of the OTL and GM diets.

The concentrate (g/kg as fed) consisted of: maize grain, 340; barley grain, 380; soybean meal, 150; wheat middlings, 110; calcium phosphate, 15; salt, 3; mineral and vitamin premix, 2. The mineral and vitamin premix contained (per kg as mixed): 150 g Ca, 100 g P, 100 g Na, 100 mg Co, 300 mg I, 5000 mg Fe, 10 000 mg Mn, 20 000 mg Zn, 100 000 mg Se, 5 000 000 i.u. retinol, 500 000 i.u. cholecalciferol and 15 000 mg α-tocopherol.

All animals were milked twice a day with a milking machine. Individual milk samples were collected from sheep and goats on DIM 125, 134, 170 and 179, after mixing the yield from the evening and the morning milking, on a volume basis of 50 ml/l.

### *Measurements and analytical methods*

Alfalfa hay, wheat straw, concentrates, OTL and GM samples were also collected and analysed for organic matter (OM; 7·009), dry matter (DM; 7·007), N (7·016) and ether extract (7·060) according to AOAC (1984) and for neutral detergent fibre (NDF) assayed without a heat stable amylase and expressed inclusive of residual ash and acid detergent fibre (ADF) expressed exclusive of residual ash according to Van Soest et al. (1991). Milk was analysed for fat and protein with IR spectrometry (Milkoscan 133/; Foss Electric, Hillerød, Demark) after appropriate calibration of the instrument according to Gerber (BSI, 1955) and Kjeldahl (IDF, 1993). Milk samples, OTL and GM were analysed for fatty acid methyl esters by GC as described by Tsiplakou et al. (2006a).

### *Calculations*

The different groups of fatty acids were defined as follows according to Stockdale et al. (2003):

Short-chain saturated fatty acids (SC-SFA)=6:0+8:0+10:0+11:0,
Medium-chain saturated fatty acids (MC-SFA)=12:0+13:0+14:0+15:0,
Long-chain saturated fatty acids (LC-SFA)=16:0 +18:0+20:0+22:0+23:0+24:0,
Polyunsaturated fatty acids (PUFA)=*cis*-9, *trans*-11 CLA+18:2n6*c*+18:2n6*t*+18:3n3*c*+18:3n6*c*+20:2+20:3n3*c* +20:3n6*c* +20:4+20:5 +22:2,

**Table 3.** Chemical composition (g/ kg dry matter) and the main fatty acids (% of total fatty acids) of alfalfa hay, olive tree leaves, grape marc and concentrates

| | Alfalfa hay ($n$=6) | Olive tree leaves ($n$=6) | Grape marc ($n$=6) | Concentrates ($n$=6) |
|---|---|---|---|---|
| Dry matter, g/kg | 910±4·3 | 912±5·9 | 910±4·6 | 885±3·9 |
| Organic matter | 923±5·1 | 948±4·4 | 938±1·6 | 917±2·8 |
| Crude protein | 132±3·6 | 100±3·6 | 116±2·4 | 150±3·0 |
| Ether extract | 9±0·8 | 58±1·0 | 89±1·5 | 16±2·2 |
| Neutral detergent fibre | 466±4·6 | 390±3·0 | 558±1·2 | 297±4·8 |
| Acid detergent fibre | 358±2·1 | 257±2·4 | 465±1·4 | 228±5·0 |
| *Fatty acid profile* | | | | |
| 14:0 | 20·7±0·44 | 2·1±0·10 | 0·3±0·03 | 0·2±0·03 |
| 16:0 | 34·0±0·31 | 21·0±0·51 | 9·4±0·09 | 16·2±0·07 |
| 18:0 | 3·1±0·10 | 2·4±0·06 | 4·4±0·09 | 1·9±0·08 |
| 18:1 | 3·3±0·12 | 12·8±0·36 | 21·7±0·13 | 0·37±0·08 |
| 18:2n6*c* | 15·0±0·45 | 13·1±0·79 | 61·1±0·33 | 76·8±0·19 |
| 18:3n3 | 21·5±0·42 | 37·0±0·66 | 0·6±0·04 | 2·7±0·08 |

Mono-unsaturated fatty acids (MUFA)=14:1+15:1+16:1+17:1+18:1+VA+20:1,
Saturated/unsaturated ratio (S/U)=(SC-SFA+MC-SFA+LC-SFA)/(PUFA+MUFA) and
VA=*trans*-11 18:1. This value is not included in the 18:1 content.

Atherogenicity index (**AI**) was defined as (12:0+4×14:0+16:0)/(PUFA+MUFA) as described by Ulbricht & Southgate (1991). The $\Delta^{-9}$ desaturase activity indexes were calculated by the following four ratios: 14:1/14:0, 16:1/16:0, 18:1/18:0 and CLA/VA.

### *Statistical analysis*

Results are presented as least square means±SEM. Treatments effects (C *v.* OTL or C *v.* GM) on milk fatty acid profile in each animal species (sheep/goat) was tested by one-way ANOVA, using SPSS statistical package (release 9.0.0). Post-hoc tests were performed using Duncan's multiple range test and significance was set at $P<0·05$. The effect of stage of lactation (DIM=125, 134, 170 and 179) on milk fatty acid profile in each animal species was determined when consuming the C diets. Additionally, Pearson correlations were performed between milk fatty acids in each animal species and the most important, according to $R$-square and $P$-values are presented in this study.

## Results

### *Diets*

The intake of OTL, as a proportion of total DM intake, was 751 and 702 g/kg DM in sheep and goats respectively, while the intake of GM was 196 and 189 g/kg DM in sheep and goats respectively (Table 1). The chemical composition and the fatty acid profile of alfalfa hay, OTL, GM and concentrates are in Table 3. The ether extract content of the OTL and GM diets was significantly higher than the respective control diets for each animal species (Table 1).

Apart from the relatively high ether extract content of both by-products, OTL fat was high in LNA (37% of total fatty acids) and that of GM was high in LA (61·1% of total fatty acids) and 18:1 (21·7% of total fatty acids) (Table 3).

### *Milk fatty acids*

The individual fatty acid profile (% of total fatty acids) of milk from sheep and goats consuming C and OTL diets is presented in Table 4. Relative to C, OTL decreased significantly the concentrations of 6:0, 8:0, 10:0, 12:0, 14:0, 14:1, 15:0 and 16:0 in sheep milk fat whereas the concentrations of 18:1, VA, LA, LNA and *cis*-9, *trans*-11 CLA increased significantly. For goats, again relative to C, OTL decreased significantly the concentrations of 11:0, 12:0, 14:0, 14:1, 15:0 and 16:0. The opposite trend was observed with the concentrations of 18:0, 18:1 and LNA in milk of goats fed the OTL diet.

The fatty acid groups (SC-SFA, MC-SFA, LC-SFA, PUFA and MUFA) and the values of S/U and AI of in milk fat of sheep and goats fed with OTL *v.* C diets, are also presented in Table 4. Concentrations of SC-SFA, MC-SFA and LC-SFA and the S/U value decreased significantly when the sheep were fed with the OTL *v.* C diet. A similar trend was observed in milk of goats fed the OTL diet, but the results were significant only for MC-SFA and S/U ratio. On the contrary, the concentrations of PUFA and MUFA increased significantly in milk fat of sheep fed with OTL *v.* C diets but only PUFA was increased in milk fat of goats. The AI value was decreased significantly only for goats fed the OTL diet.

The individual milk fatty acid profile (% of total fatty acids) in sheep and goats fed with C and GM diets are presented in Table 5. Relative the control diet, the GM diet

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**Table 4.** The mean (±SEM) fatty acid profile (% of total fatty acids)† of milk fat from sheep and goats consuming the control and the olive tree leaves diets. Values are the mean of two different samplings at days in milk (DIM) 125 and 134

| | Sheep | | Goats | |
|---|---|---|---|---|
| *Fatty acids* | Control ($n=8$) | Olive tree leaves ($n=8$) | Control ($n=8$) | Olive tree leaves ($n=8$) |
| 6:0 | $2{\cdot}00^{a}\pm0{\cdot}276$ | $1{\cdot}13^{b}\pm0{\cdot}119$ | $2{\cdot}77\pm0{\cdot}215$ | $2{\cdot}84\pm0{\cdot}244$ |
| 8:0 | $1{\cdot}51^{a}\pm0{\cdot}256$ | $0{\cdot}72^{b}\pm0{\cdot}059$ | $2{\cdot}91\pm0{\cdot}220$ | $2{\cdot}82\pm0{\cdot}211$ |
| 10:0 | $4{\cdot}84^{a}\pm0{\cdot}960$ | $1{\cdot}97^{b}\pm0{\cdot}142$ | $10{\cdot}43\pm0{\cdot}755$ | $9{\cdot}29\pm0{\cdot}666$ |
| 11:0 | $0{\cdot}05\pm0{\cdot}022$ | $0{\cdot}00\pm0{\cdot}000$ | $0{\cdot}31^{a}\pm0{\cdot}072$ | $0{\cdot}09^{b}\pm0{\cdot}015$ |
| 12:0 | $3{\cdot}21^{a}\pm0{\cdot}499$ | $1{\cdot}71^{b}\pm0{\cdot}077$ | $5{\cdot}57^{a}\pm0{\cdot}389$ | $3{\cdot}40^{b}\pm0{\cdot}251$ |
| 13:0 | $0{\cdot}10^{b}\pm0{\cdot}14$ | $0{\cdot}30^{a}\pm0{\cdot}013$ | $0{\cdot}16^{a}\pm0{\cdot}032$ | $0{\cdot}05^{b}\pm0{\cdot}011$ |
| 14:0 | $10{\cdot}07^{a}\pm1{\cdot}16$ | $5{\cdot}39^{b}\pm0{\cdot}351$ | $11{\cdot}11^{a}\pm0{\cdot}499$ | $7{\cdot}70^{b}\pm0{\cdot}214$ |
| 14:1 | $0{\cdot}37^{a}\pm0{\cdot}049$ | $0{\cdot}09^{b}\pm0{\cdot}012$ | $0{\cdot}26^{a}\pm0{\cdot}045$ | $0{\cdot}08^{b}\pm0{\cdot}008$ |
| 15:0 | $1{\cdot}26^{a}\pm0{\cdot}11$ | $0{\cdot}53^{b}\pm0{\cdot}037$ | $1{\cdot}37^{a}\pm0{\cdot}171$ | $0{\cdot}55^{b}\pm0{\cdot}033$ |
| 15:1 | $0{\cdot}40\pm0{\cdot}051$ | $0{\cdot}32\pm0{\cdot}057$ | $0{\cdot}20\pm0{\cdot}030$ | $0{\cdot}11\pm0{\cdot}066$ |
| 16:0 | $27{\cdot}41^{a}\pm1{\cdot}002$ | $20{\cdot}29^{b}\pm0{\cdot}314$ | $29{\cdot}82^{a}\pm2{\cdot}251$ | $22{\cdot}45^{b}\pm0{\cdot}567$ |
| 16:1 | $0{\cdot}59\pm0{\cdot}298$ | $0{\cdot}39\pm0{\cdot}047$ | $1{\cdot}14\pm0{\cdot}257$ | $0{\cdot}78\pm0{\cdot}078$ |
| 17:1 | $0{\cdot}38\pm0{\cdot}123$ | $0{\cdot}21\pm0{\cdot}106$ | $0{\cdot}35\pm0{\cdot}073$ | $0{\cdot}20\pm0{\cdot}038$ |
| 18:0 | $9{\cdot}74\pm0{\cdot}941$ | $8{\cdot}09\pm0{\cdot}269$ | $6{\cdot}68^{b}\pm1{\cdot}177$ | $12{\cdot}00^{a}\pm1{\cdot}173$ |
| 18:1 | $26{\cdot}89^{b}\pm2{\cdot}70$ | $35{\cdot}03^{a}\pm1{\cdot}953$ | $16{\cdot}90^{b}\pm1{\cdot}504$ | $22{\cdot}72^{a}\pm1{\cdot}167$ |
| 18:1 *trans-11*, VA‡ | $3{\cdot}89^{b}\pm0{\cdot}365$ | $8{\cdot}46^{a}\pm1{\cdot}664$ | $4{\cdot}25\pm1{\cdot}363$ | $4{\cdot}35\pm1{\cdot}044$ |
| 18:2n6*c* | $3{\cdot}47^{b}\pm0{\cdot}658$ | $7{\cdot}70^{a}\pm0{\cdot}653$ | $3{\cdot}45\pm0{\cdot}428$ | $4{\cdot}71\pm0{\cdot}285$ |
| 18:2n6*t* | $0{\cdot}12\pm0{\cdot}021$ | $0{\cdot}19\pm0{\cdot}011$ | $0{\cdot}05\pm0{\cdot}015$ | $0{\cdot}05\pm0{\cdot}020$ |
| 18:3n6 | $0{\cdot}18\pm0{\cdot}026$ | $0{\cdot}18\pm0{\cdot}022$ | $0{\cdot}13\pm0{\cdot}011$ | $0{\cdot}19\pm0{\cdot}012$ |
| 18:3n3 | $0{\cdot}36^{b}\pm0{\cdot}017$ | $1{\cdot}05^{a}\pm0{\cdot}073$ | $0{\cdot}35^{b}\pm0{\cdot}063$ | $1{\cdot}34^{a}\pm0{\cdot}029$ |
| CLA *c*-9 *t*-11 | $1{\cdot}97^{b}\pm0{\cdot}182$ | $3{\cdot}37^{a}\pm0{\cdot}415$ | $1{\cdot}00\pm0{\cdot}188$ | $1{\cdot}21\pm0{\cdot}243$ |
| SC-saturated§ | $8{\cdot}40^{a}\pm1{\cdot}500$ | $3{\cdot}81^{b}\pm0{\cdot}311$ | $16{\cdot}41\pm1{\cdot}058$ | $15{\cdot}03\pm1{\cdot}019$ |
| MC-saturated | $14{\cdot}64^{a}\pm1{\cdot}699$ | $7{\cdot}65^{b}\pm0{\cdot}426$ | $18{\cdot}22^{a}\pm0{\cdot}950$ | $11{\cdot}69^{b}\pm0{\cdot}441$ |
| LC-saturated | $37{\cdot}42^{a}\pm0{\cdot}875$ | $28{\cdot}76^{b}\pm0{\cdot}634$ | $36{\cdot}68\pm1{\cdot}605$ | $34{\cdot}98\pm1{\cdot}044$ |
| PUFA | $6{\cdot}80^{b}\pm0{\cdot}569$ | $13{\cdot}42^{a}\pm0{\cdot}621$ | $5{\cdot}37^{b}\pm0{\cdot}440$ | $8{\cdot}34^{a}\pm0{\cdot}518$ |
| MUFA | $32{\cdot}70^{b}\pm2{\cdot}832$ | $45{\cdot}01^{a}\pm0{\cdot}671$ | $23{\cdot}21\pm2{\cdot}046$ | $28{\cdot}58\pm1{\cdot}170$ |
| Saturated/unsaturated | $1{\cdot}70^{a}\pm0{\cdot}214$ | $0{\cdot}69^{b}\pm0{\cdot}032$ | $2{\cdot}68^{a}\pm0{\cdot}335$ | $1{\cdot}71^{b}\pm0{\cdot}125$ |
| AI¶ | $0{\cdot}36\pm0{\cdot}004$ | $0{\cdot}26\pm0{\cdot}007$ | $0{\cdot}62^{a}\pm0{\cdot}003$ | $0{\cdot}50^{b}\pm0{\cdot}005$ |
| CLA *c*-9 *t*-11/VA | $0{\cdot}51\pm0{\cdot}032$ | $0{\cdot}46\pm0{\cdot}088$ | $0{\cdot}52\pm0{\cdot}169$ | $0{\cdot}33\pm0{\cdot}037$ |
| 14:1/14:0 | $0{\cdot}04^{a}\pm0{\cdot}004$ | $0{\cdot}02^{b}\pm0{\cdot}002$ | $0{\cdot}02\pm0{\cdot}004$ | $0{\cdot}01\pm0{\cdot}001$ |
| 16:1/16:0 | $0{\cdot}02\pm0{\cdot}001$ | $0{\cdot}02\pm0{\cdot}003$ | $0{\cdot}04\pm0{\cdot}009$ | $0{\cdot}04\pm0{\cdot}003$ |
| 18:1/18:0 | $2{\cdot}87^{b}\pm0{\cdot}274$ | $4{\cdot}34^{a}\pm0{\cdot}564$ | $2{\cdot}87\pm1{\cdot}063$ | $2{\cdot}03\pm0{\cdot}667$ |

† Means with different superscripts (a, b) in each row between Control and Olive tree leaves treatments, for each fatty acid within an animal species differ significantly ($P<0{\cdot}05$)

‡ VA, vaccenic acid

§ SC-saturated, short-chain saturated fatty acids; MC-saturated, medium-chain saturated fatty acids; LC-saturated, long-chain saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, mono-unsaturated fatty acids

¶ AI, atherogenicity index (see text)

decreased significantly the concentrations of 11:0, 13:0, 14:0 and 16:0 in sheep milk fat. In addition, the GM diet increased significantly the concentrations of 18:0, VA, LA and *cis*-9, *trans*-11 CLA in sheep milk fat.

The fatty acid groups and the values of S/U and AI of milk fat in sheep and goats fed with GM *v.* C diets are also presented in Table 5. Concentrations of MC-SFA decreased significantly while the concentrations of PUFA increased significantly only for sheep fed with GM diets. The GM *v.* C diet in goats had no significant effect on fatty acid profile (apart from 13:0), on fatty acid groups or on S/U, AI and $\Delta^{-9}$ desaturase activity indexes values.

The *trans*-10, *cis*-12 isomer of CLA was not detected in milk fat of either animal species for any of the dietary treatments. The statistical analysis of the data showed that the sampling time (stage of lactation) had no effect on the milk fatty acid profile of either animal species consuming the C diets.

### $\Delta^{-9}$ *Desaturase*

The $\Delta^{-9}$ desaturase activity indexes in both animal species fed the C, OTL and GM diets are in Tables 4 and 5. The 18:1/18:0 and *cis*-9, *trans*-11 CLA/VA $\Delta^{-9}$ desaturase activity indexes were higher than 14:1/14:0 and 16:1/16:0 for both animal species, irrespective of diet. The only significant $\Delta^{-9}$ desaturase activity index was that of 18:1/18:0 ratio in sheep, with opposite effects of OTL and GM

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**Table 5.** The mean (±SEM) fatty acid profile (% of total fatty acids)† of milk fat from sheep and goats consuming the control and the grape marc diets. Values are the means of two different samplings at days in milk (DIM) 170 and 179

| | Sheep | | Goats | |
|---|---|---|---|---|
| *Fatty acids* | Control (*n*=8) | Grape marc (*n*=8) | Control (*n*=8) | Grape marc (*n*=8) |
| 6:0 | 2·69±0·495 | 2·03±0·249 | 2·81±0·198 | 2·26±0·168 |
| 8:0 | 2·10±0·435 | 1·38±0·163 | 3·16±0·261 | 2·40±0·149 |
| 10:0 | 4·97±1·289 | 3·49±0·679 | 9·32±1·444 | 9·36±0·738 |
| 11:0 | 0·11[a]±0·039 | 0·03[b]±0·012 | 0·15±0·031 | 0·11±0·015 |
| 12:0 | 3·74±0·679 | 2·51±0·266 | 5·10±0·774 | 5·17±0·436 |
| 13:0 | 0·14[a]±0·022 | 0·08[b]±0·011 | 0·14[a]±0·020 | 0·01[b]±0·009 |
| 14:0 | 9·95[a]±1·129 | 7·30[b]±0·553 | 12·89±0·577 | 11·95±0·746 |
| 14:1 | 0·41[a]±0·046 | 0·25[b]±0·034 | 0·20±0·028 | 0·27±0·027 |
| 15:0 | 1·25±0·195 | 1·02±0·010 | 1·06±0·144 | 0·93±0·050 |
| 15:1 | 0·32±0·011 | 0·29±0·042 | 0·13±0·015 | 0·19±0·029 |
| 16:0 | 26·35[a]±0·431 | 21·62[b]±0·676 | 31·81±1·326 | 29·85±1·037 |
| 16:1 | 0·90±0·354 | 0·35±0·161 | 0·63±0·188 | 0·42±0·216 |
| 17:1 | 0·42±0·106 | 0·36±0·083 | 0·34±0·057 | 0·27±0·088 |
| 18:0 | 9·01[b]±1·110 | 14·30[a]±0·834 | 7·21±0·729 | 8·20±0·894 |
| 18:1 | 25·99±2·86 | 29·77±1·727 | 16·19±0·958 | 18·99±1·506 |
| 18:1 *trans-11*, VA‡ | 3·78[b]±0·411 | 5·36[a]±0·562 | 3·55±0·640 | 3·20±0·356 |
| 18:2n6*c* | 4·55[b]±0·390 | 5·43[a]±0·215 | 2·89±0·257 | 3·66±0·233 |
| 18:2n6*t* | 0·08±0·023 | 0·10±0·032 | 0·05±0·018 | 0·07±0·014 |
| 18:3n6 | 0·16±0·030 | 0·17±0·020 | 0·13±0·009 | 0·09±0·012 |
| 18:3n3 | 0·33±0·020 | 0·38±0·028 | 0·32±0·061 | 0·28±0·046 |
| CLA *c*-9 *t*-11 | 1·67[b]±0·13 | 2·61[a]±0·205 | 1·05±0·239 | 1·58±0·152 |
| SC-saturated§ | 9·86±1·806 | 6·93±0·960 | 15·44±1·257 | 14·12±0·730 |
| MC-saturated | 15·09[a]±1·926 | 10·92[b]±0·885 | 19·18±0·841 | 18·14±1·105 |
| LC-saturated | 35·64±0·896 | 36·20±0·843 | 39·20±0·854 | 38·23±0·833 |
| PUFA | 7·38[b]±0·510 | 9·38[a]±0·382 | 4·84±0·475 | 6·05±0·321 |
| MUFA | 31·97±2·506 | 36·52±1·712 | 21·21±1·179 | 23·42±1·442 |
| Saturated/unsaturated | 1·65±0·202 | 1·20±0·081 | 2·93±0·234 | 2·45±0·163 |
| AI¶ | 0·41±0·063 | 0·31±0·030 | 0·53±0·075 | 0·55±0·040 |
| CLA *c*-9 *t*-11/VA | 0·47±0·053 | 0·52±0·059 | 0·34±0·064 | 0·50±0·026 |
| 14:1/14:0 | 0·04±0·004 | 0·03±0·005 | 0·02±0·002 | 0·02±0·003 |
| 16:1/16:0 | 0·03±0·001 | 0·02±0·007 | 0·02±0·007 | 0·01±0·007 |
| 18:1/18:0 | 3·02[a]±0·232 | 2·03[b]±0·164 | 2·37±0·207 | 2·49±0·285 |

† Means with different superscripts (a, b) in each row between Control and Grape marc treatments, for each fatty acid within an animal species differ significantly ($P<0·05$)

‡ VA, vaccenic acid

§ SC-saturated, short-chain saturated fatty acids; MC-saturated, medium-chain saturated fatty acids; LC-saturated, long-chain saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, mono-unsaturated fatty acids

¶ AI, atherogenicity index (see text)

diets on that ratio, compared with the respective controls. The 14:1/14:0 ratio was affected significantly by the OTL diet only in sheep.

## Discussion

It is very difficult in practice, or even under experimental conditions, to have isoenergetic and isonitrogenous diets, particularly between sheep and goats, owing to their different milk yield, milk composition, lactation persistency and dietary preferences. Thus, a great effort was made to keep the differences between treatments (C *v*. OTL or C *v*. GM) in sheep and goats as small as possible, aiming at the same time to meet the animals' nutritional requirements. Therefore, a strict statistical comparison between sheep and goats was not possible. The same concerns apply to the comparison, between OTL and GM diets, their effects on milk fatty acid profile of sheep or goats, owing to their different dietary characteristics (Tables 1 and 3).

Despite the fact that the diets (C *v*. OTL and C *v*. GM) fed to each animal species were not isoenergetic and isonitrogenous (per kg DM), the average daily energy and crude protein intakes were comparable between treated and control groups for each animal species (Table 1). Thus, it would seem unlikely that these small differences in crude protein of the diets (C *v*. OTL and C *v*. GM) in each animal species would have affected the milk fatty acid profile and especially the *cis*-9, *trans*-11 CLA and VA concentrations (Leonardi et al. 2003). The small differences in energy and crude protein intakes between C *v*. OTL and C *v*. GM in each animal species were unavoidable owing to the inclusion of OTL and GM in the diets.

Further to that, the OTL and GM diets had significantly higher ether extract contents than their respective control diets (Table 1). In addition, the LNA and LA content of OTL and GM respectively was also higher than the respective control diets (Table 3). These two differences in ether extract and in LNA and LA contents of the diets should have affected the *cis*-9, *trans*-11 $C_{18:2,}$ CLA in milk fat (Nudda et al. 2006; Zhang et al. 2006).

Diet has a major effect on milk fat CLA content. Increasing the supply of LNA and LA acids in the diet is an efficient way of improving *cis*-9, *trans*-11 CLA concentration in milk fat. Many authors have reported that pasture and linseed play a key role in improving the *cis*-9, *trans*-11 CLA content of milk fat (Atti et al. 2006; Tsiplakou et al. 2006a; Chilliard et al. 2007). Pasture and linseed increase the *cis*-9, *trans*-11 CLA content of milk fat because of their high LNA (60% of total fatty acids) content (Cabbidu et al. 2005; Chilliard et al. 2007). As well as pasture and linseed, by-products such as OTL also have high LNA content (Table 3). The OTL diets increased the *cis*-9, *trans*-11 CLA content in milk fat, compared with controls, but the results were significant only for sheep (Table 4). The increase in *cis*-9, *trans*-11 CLA was 71% in sheep milk and only 21% in goats' milk compared with their respective controls, reflecting the difference in VA content (3·89 *v*. 8·46 in the C *v*. OTL-fed sheep, and 4·25 *v*. 4·35 in the C *v*. OTL-fed goats, Table 4). These results for goats' milk do not agree with those of Nudda et al. (2006) who observed increases in *cis*-9, *trans*-11 CLA of 52–67% when the goats consumed extruded linseed cake supplements. Chilliard et al. (2003) did not observe increases in the VA of the milk fat of goats given whole linseed supplements, but did find an increase of VA of 123% when free linseed oil was used.

The increase in *cis*-9, *trans*-11 CLA and VA milk fat content in sheep fed the GM diet was significantly higher by 56% and 42% respectively than controls (Table 5). The respective changes observed in *cis*-9, *trans*-11 CLA (50%) and VA (−9·8%) milk fat content in goats were not significant (Table 5). When diets are supplemented with LA-rich seeds or oil the *cis*-9, *trans*-11 CLA and VA milk fat concentrations are affected. Comparing the effects of oils and seeds on *cis*-9, *trans*-11 CLA and VA production, paradoxically fatty acids from seeds are hydrogenated more strongly to 18:0 than are those from oils, the latter being recovered more in the form of *cis*-9, *trans*-11 CLA and VA in milk (Chilliard et al. 2003). In our study the GM diets which have high LA content, gave significantly higher *cis*-9, *trans*-11 CLA and VA contents in milk fat only for sheep. In addition, the high content of 18:1 in GM (Table 3) seems to have had no effect on 18:1 content in milk fat in sheep or goats in this experiment.

The changes in milk fatty acid composition that are obtained by lipid supplementation of ruminant diets are linked to the lipid source (animal fat, plant or marine oil) and to the form in which it is presented, technological treatment and amount included in the diet (Chilliard et al. 2007). However, the responses are also largely dependent on both the forage source and the diet forage/concentrate ratio (Chilliard & Ferlay, 2004).

The 18:0 concentration in milk fat of sheep fed the GM diet showed a sharp increase and was significantly higher than the respective C diet, while there was no difference for C *v*. OTL diets (Tables 4 and 5). On the contrary, the 18:0 concentration in milk fat of goats fed with OTL diet was significantly higher than for the respective C diet, with no significant difference between C and GM diets (Tables 4 and 5). It seems likely that OTL or GM diets may have different effects on the rumen environment between sheep and goats, which influence the build-up of biohydrogenation intermediates or limit the conversion of VA to 18:0. Possible factors include changes in rumen pH, type of lipid ingested and type and amount of bacteria inhabiting the rumen (Lock & Garnsworthy, 2003).

The apparent different effects of OTL and GM diets on the milk fatty acid profile in sheep and goats are difficult to explain solely on the basis of the unavoidable differences in the amounts and chemical composition (ether extracts, NDF, ADF etc.) among the diets offered. These dietary differences were unavoidable because, to maximize the relevance of the results to practice, it was a priority to have maximum consumption of OTL and GM by sheep and goats, to reflect their availability and low cost as raw feeding materials.

Further to diet effects (OTL/GM) the stage of lactation had no effect on milk fatty acid profile, since the C *v*. OTL or C *v*. GM were fed in both animal species at the same DIM. In addition to that, Tsiplakou et al. (2006b) found no effect of the stage of lactation on *cis*-9, *trans*-11 in milk fat when four sheep breeds were kept indoors and fed the same diet.

The present results for *cis*-9, *trans*-11 CLA, VA, 18:0 and 18:1/18:0 in milk fat suggest that sheep and goats responded differently when OTL or GM were included in their diets. This suggestion supports the notion of species differences already hypothesized by Jahreis et al. (1999), and highlights the need for further investigation. To test that hypothesis rigorously would require an experimental design incorporating strictly identical diets and comparable sheep and goat requirements.

### *Correlations*

A positive relationship was found between *cis*-9, *trans*-11 CLA and VA in milk of sheep fed the OTL diet: *cis*-9, *trans*-11 CLA $=1{\cdot}74+0{\cdot}22*\text{VA}$, $R^2=0{\cdot}73$ ($P<0{\cdot}05$). In addition, a positive relationship was also found between *cis*-9, *trans*-11 CLA and VA in milk of goats fed OTL: *cis*-9, *trans*-11 CLA=0·23+0·23 * VA, $R^2$=0·94 ($P$<0·001). A positive relationship between *cis*-9, *trans*-11 CLA and VA in milk fat of sheep grazing different pastures has also been reported by Cabiddu et al. (2005) ($R^2$=0·74, $P$<0·05) and Addis et al. (2005) ($R^2$=0·67, $P$<0·001) in sheep, and by Chilliard & Ferlay (2004) in goats ($r$=0·99) fed with hay-based diets with or without lipid supplementation (sunflower oil or soybeans or untreated lupin seeds or sunflower seeds). Additionally, a negative correlation between 18:0 and VA was found only in goats fed with the OTL diet: 18:0=16·42−1·01 * VA, $R^2$=0·82 ($P$<0·01), which may show that the last step of biohydrogenation in the goat rumen was more extensive than that occurring in sheep, despite the fact that the passage rate of digesta is usually higher in goats (Van Soest, 1997) and despite the fact that the goats consumed higher amounts of OTL than did the sheep. However, as mentioned above, under the constraints of the present experimental design no strict comparison of sheep and goats is possible.

### $\Delta^{-9}$ Desaturase

Most *cis*-9, *trans*-11 CLA in milk fat is of endogenous origin, synthesized via the enzyme $\Delta^{-9}$ desaturase from VA. The best indicator of $\Delta^{-9}$ desaturase activity is the 14:1/14:0 because all 14:0 in milk fat is produced by synthesis de novo in the mammary gland, whereas the other acid substrates can be absorbed from the gut (Cabiddu et al. 2005). Lock & Garnsworthy (2003) found that an increase of CLA in cow milk was related to an increase of 14:1/ 14:0 ratio. In this study no relationship between *cis*-9, *trans*-11 CLA and 14:1/ 14:0 ratio in milk fat of sheep or goats was found irrespective of diet consumed. Our results agree with those of Cabiddu et al. (2005) who found no relationship between *cis*-9, *trans*-11 CLA and 14:1/ 14:0 ratio in sheep milk fat. It was also observed that the 14:1/14:0 and 16:1/16:0 ratios in milk fat of sheep and goats fed either the OTL or the GM diets, were lower than the other two $\Delta^{-9}$ desaturase ratios (Tables 4 and 5). These results also agree with those of Addis et al. (2005). The $\Delta^{-9}$ desaturase activity indexes, expressed by the 14:1/14:0 and 16:1/16:0 ratios, are very low because only a small proportion of 14:0 and 16:0 is desaturated to 14:1 and 16:1 respectively (Chilliard et al. 2000). The discrepancy between cows and small ruminants (sheep and goats) could be due to an effect of animal species on $\Delta^{-9}$ desaturase activity in the mammary gland. In the present study a higher efficiency of $\Delta^{-9}$ desaturase activity was estimated in both animal species and with both diets (OTL and GM), on the basis of the ratio 18:1/18:0 (Tables 4 and 5). This might be explained by the fact that 18:0 is the most preferred substrate of $\Delta^{-9}$ desaturase in the mammary gland (Chilliard et al. 2000; Mosley & McGuire, 2003). However, the GM and OTL diets had opposite effects on 18:1/18:0 ratio. This interesting observation cannot be easily explained.

In the present study the *cis*-9, *trans*-11 CLA/VA ratio showed a declining tendency in both animal species fed with OTL diets. A decrease in $\Delta^{-9}$ desaturase activity in the mammary gland of goats, as indicated by the *cis*-9, *trans*-11 CLA/VA ratio, has been reported by Nudda et al. (2006) when the goats fed with extruded linseed cake which has high LNA content.

### Atherogenicity index

The AI characterizes the atherogenicity of dietary fat; fat with a higher AI value is assumed to be more detrimental to human health. In the human diet, lipids (particularly saturated fatty acids) are known to contribute to coronary disease (Williams, 2000). On the contrary, some unsaturated fatty acids in milk have a protective effect against the risk of cardiovascular disease, including *trans*-10, *cis*-12 CLA, MUFA (in particular oleic acid) and PUFA. The present study found a significant effect of the OTL diet on AI value only in goats (Table 4).

## Conclusions

When included in sheep and goat diets, OTL and GM increased significantly the concentrations of *cis*-9, *trans*-11 CLA and VA in milk fat only in sheep, showing an apparent difference in response between sheep and goats. These results support the hypothesis that there are species differences, which should be tested in further studies.

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