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Sheep and goats differences in CLA and fatty acids milk fat content in relation with mRNA stearoyl-CoA desaturase and lipogenic genes expression in their mammary gland

Eleni Tsiplakou[1]*, Emmanouil Flemetakis[2], Chrysanthi Kalloniati[2], George Papadomichelakis[1], Panagiotis Katinakis[2] and George Zervas[1]

[1] Department of Nutritional Physiology and Feeding
[2] Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, GR-11855, Athens, Greece

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An experiment was conducted with 12 lactating dairy ewes and 12 goats with the objective to determine whether, under the same dietary treatments, the differences in their fatty acid (FA) profile with emphasis on *cis-9 trans-11* CLA milk fat content, are reflected in the transcript levels of genes involved in FA and *cis-9, trans-11* CLA biosynthesis. The animals were fed with two diets (A, B) in different days of milk (DIM) due to the different milk yield, body weight etc, in order to have the same food intake and to avoid dietary effects. Diet A was fed to the animals on a group basis as it is traditionally used in practice, while diet B was chosen to avoid individual feed intake variation which is usually observed in group feeding. The results showed that there are significantly lower mRNA levels of acetyl-CoA carboxylase (ACC) in sheep mammary gland compared with those of goats, independently from the diet fed. The same trend was observed with the mRNA level of FA synthase (FAS), but the results were significant only for diet A. The mRNA level of lipoprotein lipase (LPL) in the mammary gland did not differ between sheep and goats fed with diet A. In addition, the concentration of *cis-9 trans-11* CLA content was significantly higher in sheep milk fat compared with those of goats. This is in accordance with the significant higher levels on mRNA of stearoyl-CoA desaturase (SCD) which were observed in their mammary adipocytes of sheep compared with those of goats, independently of the fed diet (A or B). In conclusion, these findings demonstrate that the differences between sheep and goats, concerning *cis-9, trans-11* CLA and FA milk fat content, under the same dietary treatments could be explained in part by the differences in mRNA of SCD and lipogenic genes in their mammary gland.

Keywords: Animal species, Lipogenic genes, Δ^{-9}-desaturase, Conjugated linoleic acid.

Abbreviations: ACC, acetyl-CoA carboxylase; FA, fatty acids; FAS, fatty acid synthase; F/C, forage/concentrate; LPL, lipoprotein lipase; LEP, leptin; SCD, stearoyl-CoA desaturase.

There is growing consumer recognition of the link between diet and health. This awareness impacts food choices and the term ''functional food'' is a generic one often used to describe this concept (Milner, 1999). The functional role of conjugated linoleic acid (CLA) has been recognized by the National Research Council (NRC, 1996). CLA is a generic term for a range of positional and geometric isomers of linoleic acid with benefits to human health. The *cis-9, trans-11* CLA is responsible for the anticarcinogenic properties of CLA although the mechanisms are still under study (Larsson et al. 2005). The *trans-12, cis-10* CLA decreases fat mass in animals, while in human studies these effects are inconclusive (Parodi, 2004).

Dairy products provide approximately 75% of human CLA dietary intake (Bauman, 2006). The *cis-9, trans-11* CLA in milk fat is the major isomer and it represents about 78–89% of the total CLA in sheep milk fat (Luna et al. 2005). To our knowledge there is no such information for goats milk. Diet (Addis et al. 2005; Nudda et al. 2006; Tsiplakou et al. 2006a; Tsiplakou & Zervas, 2008a) and season (Nudda et al. 2005; Tsiplakou et al. 2006a) have

*For correspondence; e-mail: eltsiplakou@aua.gr

major effect on CLA milk fat content of small ruminants, while other factors like breed, stage of lactation and parity have no effect on sheep (Tsiplakou et al. 2006b) or cows (Kelsey et al. 2003).

Some published results have shown that there are great differences, where CLA milk fat content is concerned, between animal species. Such differences between ruminant species have mainly been attributed to different feeding regimen. Recently, Tsiplakou and Zervas (2008a), showed that when in sheep and goats diets included olive tree leaves or grape marc, sheep milk fat had significantly higher *cis-9, trans-11* CLA and vaccenic acid (*trans-11* $C_{18:1}$, VA) concentrations compared to those of goats. The same researchers, in order to avoid the confounding effect of different food intake, fed the two animal species with the same diet (in quantity and composition) and found that there were great species differences with sheep having also significantly higher *cis-9, trans-11* CLA and VA concentrations in milk compared with those of goats (Tsiplakou & Zervas, 2008b). To our knowledge there are no published data aimed at the mechanism of these species differences when sheep and goats are fed with the same diet. Bearing in mind that the *cis-9, trans-11* CLA is produced primarily in the mammary gland by Δ^9-desaturase from VA, an intermediate formed in rumen by biohydrogenation (Bauman and Griinari, 2003), the objective of this work was to determine if the differences in the fatty acid (FA) profile, with emphasis on *cis-9, trans-11* CLA milk fat content, between sheep and goats, under the same dietary treatments are reflected in the transcript levels of lipogenic genes (ACC, FAS, LPL and SCD) involved in FA or in particular in *cis-9, trans-11* CLA biosynthesis.

Materials and Methods

The experiment was conducted according to guidelines of the Agricultural University of Athens for the care and use of farm animals by the use of proper management in order to avoid any unnecessary discomfort to the animals.

Twelve, Friesian dairy ewes, 70·1±2·53 kg average body weight (BW) of good body condition (3·0±0·14) and twelve Alpine dairy goats, 56·3±2·11 kg BW of good body condition (2·5±0·19), were used for the experiment. Lambing and kidding started in December and in February respectively, and lasted 2 weeks. All sheep and goats were at their 2nd parity.

The animals were fed with two diets (A and B), consisted of alfalfa hay, wheat straw and concentrates which were from the same batch. The concentrate diet (g/kg) consisted of: maize grain, 360; barley grain, 360; soybean meal, 160; wheat middlings, 110; calcium phosphate, 15; common salt, 3; mineral and vitamins premix, 2. The mineral and vitamin premix contained (per kg as mixed): 150 g Ca, 100 g P, 100 g Na, 100 Co, 300 mg I, 5,000 mg Fe, 10,000 mg Mn, 20,000 mg Zn, 100,000 mg Se, 5,000,000 IU retinol, 500,000 IU cholecalciferol and 15,000 mg α-tocopherol.

The chemical composition and the calculated energy content of alfalfa hay, wheat straw and concentrates are shown in Table 1.

Because the sheep and the goats had different requirements (due to different milk yield, body weight etc) (Table 2), each diet (A and B) were not offered to the animals at the same days in milk (DIM), but when the animals had about the same energy and protein requirements (Table 1). Each dietary treatment lasted 3 weeks. Water was offered *ad libitum* to animals throughout the experimental period. All ewes and goats were weighed in the first and the last day of each dietary treatment.

Diet A

Diet A was fed on a group basis as it is traditionally used in practise. Further to that, since food intake affects CLA milk fat content, this regimen was decided in order to have comparable food intake by both animal species. For this purpose Diet A was offered to sheep from DIM=105 to DIM=126, and to goats, from DIM=63 to DIM=84, in order to meet their nutritive requirements. The composition of diet A was calculated according to sheep and goats maintenance and lactation requirements (Zervas et al. 2004), taking into account their average body weight, milk yield and milk fat content. Thus, per each group of sheep and goats, diet A consisted daily of 14 kg alfalfa hay, 4 kg wheat straw and 16 kg concentrate with a F/C ratio 53/47 on dry matter (DM) basis. The forages and the concentrates were offered to animals twice a day in two equal parts, at 0800 and 1600 h. No refusals were left from the offered diet. Diet A was also the starting point of the experiment, in order to plane the diet B and the offered amount of food to be consumed by any individual sheep and/or goat.

Diet B

This dietary treatment was chosen to avoid the individual feed intake variation and selectivity, which is usually observed in group feeding, among animals of the same species or between the two animal species. All ewes and goats were fed individually, in order to have comparable energy and crude protein intake, twice a day in two equal parts at 0800 and 1600 h, with 1·0 kg alfalfa hay, 0·3 kg wheat straw and 1·34 kg concentrate daily per animal, with a F/C ratio 49/51 on DM basis. Diet B consumed by sheep from DIM=140 to DIM=161 whereas by goats from DIM=98 to DIM=119. Diet selectivity did not occur and no refusals were left from any of the animals.

The dietary treatments A and B were chosen due to their complementary properties in order to study the species (sheep/goats) differences with the minimum dietary effects. Diet A met the average groups requirements but with an individual variation in food intake among the animals,

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Table 1. Mean daily energy (MJ NEL*) and crude protein (g) requirements of each sheep and goat and their respective intakes per dietary treatment (A and B) and chemical composition (g/kg dry matter) and energy content (MJ NEL*/kg dry matter) of alfalfa hay, wheat straw and concentrate (Mean ± SEM)[1]

Treatment	Diet A		Diet B	
Animal species	S[2]	G[3]	S[2]	G[3]
DIM[4]	105–126	63–84	140–161	98–119
Requirements				
NEL* (MJ/d)	15·0	15·2	13·0	13·5
Crude protein, (g/d)	394	321	330	283
Intake				
NEL* (MJ/d)	14·8	14·8	14·0	14·0
Dry matter (kg/d)	2·53	2·53	2·35	2·35
Crude protein (g/d)	352	352	329	329
Fatty acids intake (g/d)				
$C_{14:0}$	1·91	1·91	1·64	1·64
$C_{16:0}$	6·11	6·11	5·68	5·68
$C_{18:0}$	0·58	0·58	0·55	0·55
$C_{18:1}$	0·46	0·46	0·41	0·41
$C_{18:2n6c}$	15·75	15·75	15·51	15·51
$C_{18:3n3}$	2·41	2·41	2·09	2·09

Feeds	Alfalfa hay (n = 6)	Wheat straw (n = 6)	Concentrates (n = 6)
Dry matter, g/kg	912 ± 4·1	871 ± 2·7	881 ± 4·2
Crude protein	140 ± 3·4	26 ± 1·1	165 ± 2·8
Ether extract	8·7 ± 0·9	14 ± 0·6	15·2 ± 2·0
Neutral detergent fibre	458 ± 4·0	718 ± 3·8	285 ± 5·2
Acid detergent fibre	362 ± 2·2	512 ± 2·6	234 ± 4·5
NEL* (MJ/kg dry matter)	4·45 ± 0·3	3·2 ± 0·4	7·7 ± 0·4

[1] SEM = standard error of the mean, [2]S = Sheep, [3]G = Goats, [4]DIM = Days in milk
* NEL = net energy of lactation (calculated)

Table 2. Body weight (kg), milk yield (g/day/head) and milk chemical composition (g/kg) of sheep and goat species fed either with diet A or with diet B (Mean ± SEM)[1]

	Species	Diet A	Diet B
Body weight	S[2]	70·1[a] ± 2·55	68·9[a] ± 2·20
	G[3]	56·3[b] ± 2·65	53·9[b] ± 2·70
Milk yield	S	2040·0[a] ± 28·83	1541·7[a] ± 30·73
	G	3001·8[b] ± 163·10	2231·8[b] ± 2·40
Fat	S	66·0[a] ± 2·76	70·4[a] ± 2·77
	G	40·2[b] ± ·68	50·1[b] ± 1·34
Protein	S	56·2[a] ± 1·82	55·75[a] ± 1·58
	G	32·56[b] ± 0·85	35·1[b] ± 0·56
Lactose	S	50·9[a] ± 0·56	52·1[a] ± 0·69
	G	47·2[b] ± 0·59	50·1[b] ± 0·56
Solids not fat	S	126·2[a] ± 1·35	131·1[a] ± 1·64
	G	121·2[b] ± 1·45	110·6[b] ± 1·85
Total solids	S	192·2[a] ± 3·36	201·5[a] ± 3·52
	G	161·4[b] ± 6·21	160·7[b] ± 3·99

Superscripts [1, 2 and 3] as in Table 1
Means with different superscripts (a, b) for each parameter in each column (between sheep and goats at each dietary treatment) differ significantly ($P \leqslant 0{\cdot}05$)

while with diet B all the animals fed individually with the same amount of food, despite the fact that some animals did not cover their requirements.

Measurements and sample collection

All animals were milked twice a day by milking machine. Two individual milk samples were taken from sheep and goats on the 15th and the last day (21st) of each dietary treatment, after mixing the yield from the evening and the morning milking, on a percent volume (5%). Blood samples were also taken on the last day of each dietary treatment from the jugular vein into heparin-containing tubes and subsequently centrifuged at 2700 ***g*** for 15 min to separate plasma from the cells.

Mammary adipocytes (MA) were also taken by biopsy on the last day of each dietary treatment after morning milking. Before the biopsy the mammary gland of the animals was shaved and cleaned. A 2–3 cm incision with scalpel was made in the skin on the upper portion of the right udder in order to reach adipocytes. About 1 g MA was collected under sterile conditions and immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

Analyses

Milk and feed samples were analysed for FA profile by gas chromatography, as described by Tsiplakou et al. (2006a). The plasma FA analysis was done according to the method

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Table 3. Primers used for real-time RT-qPCR

Gene	Acc. No.	Forward primer	Reverse primer	Amplicon size (bp)
OaSCD	AJ001048	5′-TTCTCTTTCTCCTCATTGCCCC-3′	5′-TCGGCTTTGGAAGCTGGAA-3′	72
ChSCD	AF325499	5′-TGTCCACCATGAACCACGTGT-3′	5′-CCACCCCTTAGCTGATGCATT-3′	89
OaLPL	NM_001009394	5′-TACCCTAACGGAGGCACTTTCC-3′	5′-TGCAATCACACGGAGAGCTTC-3′	62
ChLPL	DQ370053	5′-TACCCTAACGGAGGCACTTTCC-3′	5′-TGCAATCACACGGAGAGCTTC-3′	62
OsFAS	AF479289	5′-AAGAGAAGCTGCAGGCCAGTGT-3′	5′-CCAATTTCCAGGAATCGACCAT-3′	60
ChFAS	DQ915966	5′-TGGTGATGAACGTCTACCGTGA-3′	5′-GGACGTTTATGAAGGCGTGCT-3′	104
OaACC	NM_001009256	5′-CCGAACTGCGACTCGTTAAAT-3′	5′-CGGAGAGTGAGCATCACTGACT-3′	60
ChACC	DQ370054	5′-TCTTTGGCCTACGACGAGATCA-3′	5′-AGGTAAGCCCCAATCCCAATG-3′	70
OaLEP	U84247	5′-ATGGACCAGACATTGGCAATCT-3′	5′-GGATCACATTTCTGGAAGGCAG-3′	63
ChLEP	AM114397	5′-TGGTTTGGACTTCATCCCTGG-3′	5′-CCAATGTCTGGTCCATCTTGGA-3′	64
OaGAPDH	AF030943	5′-TGTCCGTTGTGGATCTGACCT-3′	5′-CCTGCTTCACCACCTTCTTGAT-3′	74
ChGAPDH	AJ431207	5′-AAAGGCCATCACCATCTTCCA-3′	5′-ACCACGTACTCAGCACCAGCAT-3′	74

of Bondia-Pons et al. (2004). Alfalfa hay, wheat straw and concentrates were analysed for their chemical composition as described by Tsiplakou et al. (2008b). Plasma non-esterified fatty acids (NEFA) concentrations were determined by spectrophotometric assays using kit C, WAKO, Unipath S.A., Dardilly, France.

Determination of transcripts levels using real-time RT-qPCR assay

Total RNA was isolated from 1 g MA using the Trizol reagent (Invitrogen, Paisley, UK) according to the manufacturer protocol. Prior to RT-PCR, the total RNA samples were treated with DNase I (Promega, Madison, WI) at 37 °C for 60 min. The complete digestion of genomic DNA was confirmed by real-time PCR reaction using our gene specific primers. First-strand cDNA was reverse transcribed from 2 μg of DNase-treated total RNA, using SuperScript II reverse transcriptase (Invitrogen), according to standard protocols. The resulted first-strand cDNA was diluted to a final volume of 100 μl, and SYBR green-labelled PCR fragments were amplified using gene-specific primers (Table 3) designed from the transcribed region of each gene using Primer Express 1.5 software (Applied Biosystems, Darmstadt, DE). RT-PCR reactions were performed on the Stratagene MX3005P real-time PCR apparatus using Power-SYBR Green master mix (Applied Biosystems) at a final volume of 15 μl, gene-specific primers at a final concentration of 0·2 μM each and 1 μl of the cDNA as template. PCR cycling started at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. The primer specificity and the formation of primer-dimmers were monitored by dissociation curve analysis and agarose gel electrophoresis. The expression levels of GAPDH were used as internal standards. The use of GAPDH as an internal standard was validated as in all our samples GAPDH transcript levels remained relatively constant with Ct values ranging between 20 and 21·5. Relative transcript levels of the gene of interest (X) were calculated as a ratio to the GAPDH gene transcripts (C), as $(1+E)^{-\Delta Ct}$, where ΔC_t was calculated as $(C_t^X - C_t^C)$. PCR efficiency (E) for each amplicon was calculated employing the linear regression method on the Log (Fluorescence) per cycle number data, using the LinRegPCR software (Ramakers et al. 2003).

Statistical analysis

Milk data were analysed using the general linear model (GLM) repeated measures analysis of variance (ANOVA) procedure in order to assess the effects of species (i.e sheep and goats), diet (A & B) and their interactions (species × diet) using the SPSS statistical package program, version 8.0.0. The model used was: $Y_{ij}=\mu+S_i+P_j+(S\times P)_{ij}+e_{ij}$ where Y_{ij}=dependent observation; μ=overall mean; S_i=effect of species (i=sheep, goats); P_j=effect of diet (j=A & B); $(S\times P)_{ij}$=effect of interaction between species and diet; and e_{ij}=the residual error. Diet was used as the factor indicating the repetition within the experimental unit. The animal was used in the model as a random factor. In addition, within each dietary treatment differences between the species were analysed using GLM general factorial ANOVA.

The effects of diet (A & B) in each animal species (i.e sheep and goats) or the effects of species in each dietary treatment on the transcripts levels of the genes was tested using Student's t test. Statistical significance was determined at an alpha level of $P<0{\cdot}05$.

Results and Discussion

Within the mammary gland, the metabolic pathway for *de novo* FA synthesis involves two key enzymes: ACC and FAS, while LPL is involved in the uptake of plasma FA. These FA could then be desaturated by SCD resulting in synthesis of *cis-9* unsaturated FA. In the mammary gland both epithelium and the mammary adipocytes (MA) optimize their metabolism for the synthesis and utilization of milk FA in both independent and interdependent manners

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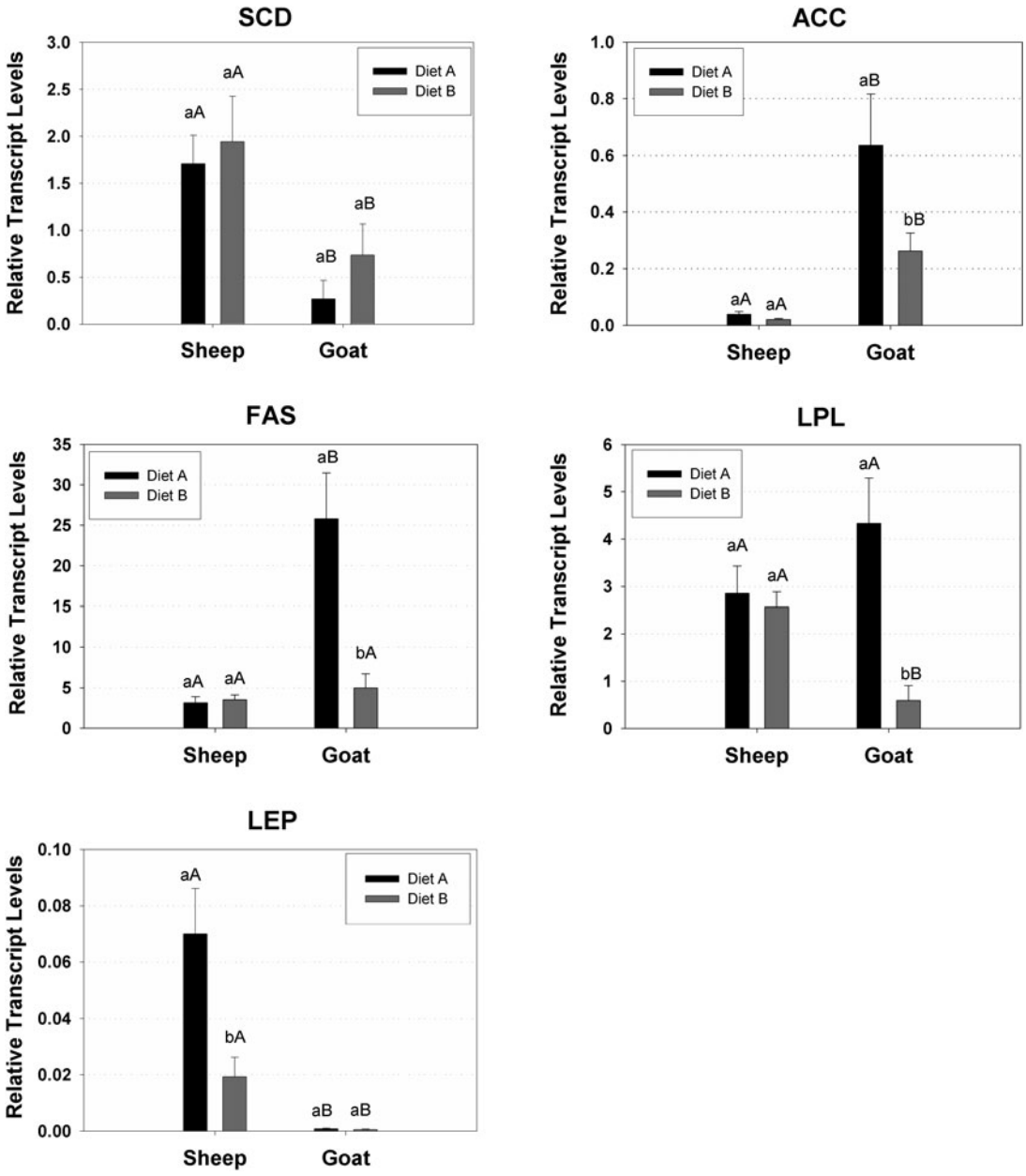

Fig. 1. Transcript accumulation of genes involved in *cis-9, trans-11* $C_{18:2}$ CLA and FA biosynthesis. SCD = Stearoyl-CoA desaturase, ACC = Acetyl-CoA carboxylase, FAS = Fatty acid synthase, LPL = Lipoprotein lipase, LEP = Leptin.

Superscripts with small letters (a,b) in each animal species (sheep/goats) between the two dietary treatments (A/B) differ significantly ($P \leqslant 0{\cdot}05$)
Superscripts with capital letters (A,B) between the two animal species (sheep/goats) in each dietary treatment (A/B) differ significantly ($P \leqslant 0{\cdot}05$)

using many of the same enzymes encoded by the same genes (Rudolph et al. 2007).

In the present study significant reduction on mRNA of ACC and FAS in the MA of goats (Fig. 1) was observed between the two dietary treatments, which is in accordance with the significant reduction of $C_{14:0}$ and $C_{16:1}$ in their milk fat content, while the decrease in $C_{16:0}$ concentration was not significant (Table 4). The close relationship between mRNA of ACC and FAS and short- and medium-chain FA has already been observed in cows by Piperova et al. (2000), using a milk fat depressing diet (25/70% forage /concentrate, supplemented with 5% soybean oil) and Ahnadi et al. (2002), using diet supplemented with fish oil, and in goats by Bernard et al. (2005b) using hay- based diet supplemented with formaldehyde-treated linseed. From the above results of cows and goats,

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Table 4. Individual fatty acids profile (% of total FA), Δ^{-9} desaturase ratios concentrations of sheep and goat milk fat fed either with diet A or B (Mean ± SEM)[1]

		Diets		Effects		
	Species	A	B	Species	Diets	Species × Diets
$C_{6:0}$	S[2]	$2{\cdot}86^{a} \pm 0{\cdot}101$	$2{\cdot}67^{bA} \pm 0{\cdot}118$	NS	NS	**
	G[3]	$2{\cdot}84^{a} \pm 0{\cdot}084$	$3{\cdot}06^{bB} \pm 0{\cdot}078$			
$C_{8:0}$	S	$2{\cdot}61 \pm 0{\cdot}144$	$2{\cdot}46^{A} \pm 0{\cdot}145$	**	NS	**
	G	$2{\cdot}96^{a} \pm 0{\cdot}136$	$3{\cdot}27^{bB} \pm 0{\cdot}120$			
$C_{10:0}$	S	$8{\cdot}49^{A} \pm 0{\cdot}508$	$7{\cdot}91^{A} \pm 0{\cdot}512$	**	NS	**
	G	$10{\cdot}32^{aB} \pm 0{\cdot}535$	$11{\cdot}19^{bB} \pm 0{\cdot}396$			
$C_{11:0}$	S	$0{\cdot}11 \pm 0{\cdot}028$	$0{\cdot}07 \pm 0{\cdot}047$	NS	NS	NS
	G	$0{\cdot}09 \pm 0{\cdot}010$	$0{\cdot}09 \pm 0{\cdot}012$			
$C_{12:0}$	S	$4{\cdot}97 \pm 0{\cdot}302$	$4{\cdot}73 \pm 0{\cdot}292$	NS	NS	NS
	G	$4{\cdot}17 \pm 0{\cdot}238$	$4{\cdot}39 \pm 0{\cdot}239$			
$C_{13:0}$	S	$0{\cdot}10 \pm 0{\cdot}015$	$0{\cdot}09 \pm 0{\cdot}007$	NS	NS	NS
	G	$0{\cdot}08 \pm 0{\cdot}005$	$0{\cdot}08 \pm 0{\cdot}007$			
$C_{14:0}$	S	$12{\cdot}02^{aA} \pm 0{\cdot}378$	$11{\cdot}18^{bA} \pm 0{\cdot}387$	**	***	NS
	G	$10{\cdot}49^{aB} \pm 0{\cdot}453$	$9{\cdot}69^{bB} \pm 0{\cdot}321$			
$C_{14:1}$	S	$0{\cdot}28 \pm 0{\cdot}018$	$0{\cdot}30^{A} \pm 0{\cdot}020$	**	NS	NS
	G	$0{\cdot}23 \pm 0{\cdot}027$	$0{\cdot}19^{B} \pm 0{\cdot}017$			
$C_{15:0}$	S	$1{\cdot}76 \pm 0{\cdot}694$	$1{\cdot}06^{A} \pm 0{\cdot}036$	NS	NS	NS
	G	$0{\cdot}96^{a} \pm 0{\cdot}051$	$0{\cdot}88^{Bb} \pm 0{\cdot}048$			
$C_{15:1}$	S	$0{\cdot}15^{A} \pm 0{\cdot}009$	$0{\cdot}16^{A} \pm 0{\cdot}017$	***	**	***
	G	$0{\cdot}11^{aB} \pm 0{\cdot}016$	$0{\cdot}02^{bB} \pm 0{\cdot}002$			
$C_{16:0}$	S	$27{\cdot}20^{aA} \pm 0{,}561$	$25{\cdot}42^{bA} \pm 0{\cdot}605$	**	**	NS
	G	$30{\cdot}36^{B} \pm 0{\cdot}999$	$29{\cdot}24^{B} \pm 0{\cdot}904$			
$C_{16:1}$	S	$1{\cdot}32^{a} \pm 0{\cdot}047$	$1{\cdot}45^{bA} \pm 0{\cdot}048$	***	***	***
	G	$1{\cdot}05^{a} \pm 0{\cdot}125$	$0{\cdot}11^{bB} \pm 0{\cdot}013$			
$C_{17:1}$	S	$0{\cdot}30^{a} \pm 0{\cdot}012$	$0{\cdot}38^{bA} \pm 0{\cdot}018$	*	**	***
	G	$0{\cdot}39^{a} \pm 0{\cdot}084$	$0{\cdot}03^{bB} \pm 0{\cdot}004$			
$C_{18:0}$	S	$8{\cdot}03 \pm 0{\cdot}374$	$7{\cdot}84 \pm 0{\cdot}306$	NS	**	*
	G	$8{\cdot}89^{a} \pm 0{\cdot}634$	$7{\cdot}71^{b} \pm 0{\cdot}544$			
$C_{18:1}$	S	$18{\cdot}63^{a} \pm 0{\cdot}803$	$22{\cdot}40^{bA} \pm 0{\cdot}928$	NS	*	**
	G	$19{\cdot}74 \pm 1{\cdot}265$	$18{\cdot}97^{B} \pm 0{\cdot}709$			
VA	S	$3{\cdot}19^{A} \pm 0{\cdot}182$	$3{\cdot}49^{A} \pm 0{\cdot}150$	***	**	NS
	G	$1{\cdot}79^{aB} \pm 0{\cdot}184$	$2{\cdot}61^{bB} \pm 0{\cdot}196$			
cis-9, trans-11 $C_{18:2}$ CLA	S	$1{\cdot}32^{aA} \pm 0{\cdot}930$	$1{\cdot}69^{bA} \pm 0{\cdot}115$	***	***	NS
	G	$0{\cdot}82^{aB} \pm 0{\cdot}073$	$1{\cdot}16^{bB} \pm 0{\cdot}093$			
$C_{18:2n6c}$	S	$4{\cdot}27^{A} \pm 0{\cdot}263$	$4{\cdot}48 \pm 0{\cdot}438$	*	*	NS
	G	$3{\cdot}20^{aB} \pm 0{\cdot}165$	$3{\cdot}99^{b} \pm 0{\cdot}213$			
$C_{18:2n6t}$	S	$0{\cdot}50^{aA} \pm 0{\cdot}030$	$0{\cdot}36^{b} \pm 0{\cdot}044$	**	NS	*
	G	$0{\cdot}30^{B} \pm 0{\cdot}018$	$0{\cdot}33 \pm 0{\cdot}027$			
$C_{18:3n6}$	S	$0{\cdot}14 \pm 0{\cdot}009$	$0{\cdot}15 \pm 0{\cdot}003$	NS	NS	NS
	G	$0{\cdot}14 \pm 0{\cdot}011$	$0{\cdot}13 \pm 0{\cdot}011$			
$C_{18:3n3}$	S	$0{\cdot}60^{aA} \pm 0{\cdot}045$	$0{\cdot}44^{b} \pm 0{\cdot}036$	**	NS	***
	G	$0{\cdot}30^{aB} \pm 0{\cdot}025$	$0{\cdot}46^{b} \pm 0{\cdot}039$			
$C_{14:1}/C_{14:0}$	S	$0{\cdot}024^{a} \pm 0{\cdot}002$	$0{\cdot}027^{bA} \pm 0{\cdot}002$	NS	NS	NS
	G	$0{\cdot}023 \pm 0{\cdot}004$	$0{\cdot}019^{B} \pm 0{\cdot}002$			
$C_{16:1}/C_{16:0}$	S	$0{\cdot}048^{aA} \pm 0{\cdot}002$	$0{\cdot}057^{bA} \pm 0{\cdot}002$	***	NS	*
	G	$0{\cdot}036^{B} \pm 0{\cdot}005$	$0{\cdot}034^{B} \pm 0{\cdot}003$			
$C_{18:1}/C_{18:0}$	S	$2{\cdot}33^{a} \pm 0{\cdot}061$	$2{\cdot}88^{b} \pm 0{\cdot}113$	NS	***	*
	G	$2{\cdot}31^{a} \pm 0{\cdot}193$	$2{\cdot}59^{b} \pm 0{\cdot}203$			
cis-9, trans-11 $C_{18:2}$ CLA/VA	S	$0{\cdot}42^{a} \pm 0{\cdot}017$	$0{\cdot}48^{b} \pm 0{\cdot}028$	NS	NS	*
	G	$0{\cdot}48 \pm 0{\cdot}033$	$0{\cdot}45 \pm 0{\cdot}037$			

Superscripts [1, 2 and 3] as in Table 1

VA = trans-11 $C_{18:1}$. This value is not included in the $C_{18:1}$ content.

Means with different superscripts with small letters (a,b) in each row for each parameter in sheep and goats (between the dietary treatments) differ significantly ($P \leqslant 0{\cdot}05$)

Means with different superscripts with capital letters (A,B) in each column for each parameter (between sheep and goats in each dietary treatment) differ significantly ($P \leqslant 0{\cdot}05$)

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the accumulation of ACC transcripts and the $C_{4:0} - C_{16:0}$ milk FA were positively related (r = +0·66) (Bernard et al. 2006). However, the concentrations of $C_{6:0}$, $C_{14:0}$, and $C_{16:0}$ in sheep milk fat decreased significantly when the animals were fed with diet B compared with diet A, even though the mRNA of ACC and FAS in the MA did not follow this reduction and the FA intake was comparable between the two diets (Table 1).

In addition, significant reduction was also observed in mRNA levels of LPL in the MA of goats fed with diet B compared with those of diet A. Due to the higher individual milk yield variation among the goats compared with sheep, diet B caused negative energy balance for some of the goats which affected their body weight, but not significantly (Table 2). These results agree with those by Jensen et al. (1994), who showed that underfeeding down regulates the expression of LPL gene in the mammary gland of mouse. The same trend, concerning LPL gene expression, has been also observed and by Bonnet et al. (2000) in adipose tissue and cardiac muscle of sheep. Furthermore, the significant reduction in mRNA levels of LPL in the MA of goats, fed with diet B, compared with diet A, is in accordance with the significant reduction of $C_{18:0}$ in their milk fat, even though their $C_{18:0}$ blood plasma concentration did not differ between the two dietary treatments (Table 5). The $C_{18:0}$ milk FA is imported from the plasma where it is released by the enzyme LPL (Barber et al. 1997) from triglycerides circulating in chylomicra or in very low density lipoproteins. The concentration of $C_{18:0}$ in sheep milk fat and blood plasma did not differ between the two dietary treatments (Tables 4 and 5). Interestingly, no significant changes on LPL transcript levels were observed in sheep between the two dietary treatments (Fig. 1).

The very low accumulation of LEP transcripts in the MA of goats, independently of the dietary treatments (Fig. 1), could be explained by the fact that during lactation adipocytes were decreased dramatically in the mammary gland as it has been shown by Bonnet et al. (2002) in sheep mammary gland. The higher mRNA of LEP sheep MA fed with diet A compared with diet B (Fig. 1) could be attributed to the progress of lactation since the FA intake between the two diets were comparable (Table 1). These results are in accordance with those by Vernon et al. (2002) who showed that the mRNA of LEP decreased during lactation in rodents. The difference on mRNA of LEP in the MA between the two animal species, independently of the fed diet (Fig. 1) could be explained by the different body condition score which was much higher in sheep compared to goats.

The sheep did not show any significant differences in ACC, FAS and LPL gene expression in the MA between the two dietary treatments (Fig. 1), even though there were at different DIM (stage of lactation). This was not the case with goats which showed significant reduction in ACC, FAS and LPL gene expression in the MA from diet A to diet B. Miller et al. (2006) has shown in dairy cows that the stage of lactation had no effect on ACC, FAS and LPL gene expression, which are related to milk synthesis. This supports the hypothesis that other factors, further to stage of lactation, may affect those genes regulation. Probably the negative energy balance of goats fed the diet B (Table 1) could be partly responsible for the above genes expression reduction. This negative energy balance of goats was rather unavoidable, in contrast to sheep, because they had high individual milk yield variation among them and the objective of this study was each of them to consume the same amount of food. The same food and FA intake, by each sheep and goat, was chosen in order to avoid dietary effects which are the major factor affecting the FA profile of milk and blood plasma. Thus, the choice of both diets (A and B) was to minimize the effect of diet and to underline species effects on genes mRNA expression and on VA and *cis-9, trans-11* CLA milk fat content.

In sheep, the milk fat concentrations of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{16:0}$ were significantly lower compared with those of goats when the animals were fed with diet B (Table 4). The same pattern was observed with diet A, only for the concentrations of $C_{10:0}$ and $C_{16:0.}$ These results could again be explained by changes in mRNA of ACC and FAS levels. Indeed, the mRNA of ACC was significantly lower in sheep MA compared with that of goats independently of the fed diet (Fig. 1). The same trend was observed in the mRNA of FAS, but the results where significant only for diet A. The non statistical differences in $C_{18:0}$ milk fat and blood plasma concentration between the two animal species agrees with the results on mRNA of LPL in the MA, when the animals were fed with diet A. When the animals were fed with diet B the gene expression of LPL differed significantly between the two animal species even though there was non statistical difference in $C_{18:0}$ milk and blood plasma. This fact could again be explained by the negative energy balance of goats compared with sheep when the animals were fed with diet B.

When the animals were fed either with diet A or B the main FA in their milk fat were $C_{16:0}$ and $C_{18:1}$, with $C_{16:0}$ having the highest concentration in both animal species, followed by, in decreasing order, $C_{10:0}$, $C_{14:0}$, $C_{18:0}$, $C_{12:0}$, $C_{18:2n6c}$, $C_{8:0}$, $C_{6:0}$, and $C_{15:0}$ (Table 4). However, some other FA identified in milk fat showed individual content less than 1% in both animal species (data are not shown).

In both sheep (Ward et al. 1998) and goats (Bernard et al. 2001) genomes, SCD is encoded by a single gene, which is transcribed in a 5-kb mRNA. The SCD gene is highly expressed in lactating mammary gland of goats (Bernard et al. 2005a) and sheep (Ward et al. 1998). Studies on SCD gene expression in sheep and goats mammary gland are limited. In ruminants, the only available data concern the nutritional regulation of SCD gene expression both *in vivo* and *in vitro*. Few *in vivo* trials have been carried out in mid-lactation cows (Piperova et al.

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Table 5. Individual fatty acids profile (% of total FA), and non esterified fatty acids (NEFA) (mg/dL) concentrations of blood plasma of sheep and goat milk fat fed either with diet A or B (Mean ± SEM)[1]

	Species	Diets		Effects		
		A	B	Species	Diets	Diets × Species
$C_{14:0}$	S[2]	$0{\cdot}52^{a} \pm 0{\cdot}033$	$0{\cdot}45^{b} \pm 0{\cdot}026$	NS	**	NS
	G[3]	$0{\cdot}53^{a} \pm 0{\cdot}040$	$0{\cdot}39^{b} \pm 0{\cdot}020$			
$C_{14:1}$	S	$0{\cdot}19^{a} \pm 0{\cdot}018$	$0{\cdot}12^{b} \pm 0{\cdot}019$	NS	***	NS
	G	$0{\cdot}18^{a} \pm 0{\cdot}028$	$0{\cdot}11^{b} \pm 0{\cdot}019$			
$C_{15:0}$	S	$0{\cdot}55^{a} \pm 0{\cdot}030$	$0{\cdot}45^{b} \pm 0{\cdot}021$	NS	**	NS
	G	$0{\cdot}61^{a} \pm 0{\cdot}059$	$0{\cdot}45^{b} \pm 0{\cdot}023$			
$C_{15:1}$	S	$0{\cdot}14^{A} \pm 0{\cdot}023$	$0{\cdot}13^{A} \pm 0{\cdot}021$	**	NS	NS
	G	$0{\cdot}25^{B} \pm 0{\cdot}027$	$0{\cdot}20^{B} \pm 0{\cdot}016$			
$C_{16:0}$	S	$28{\cdot}73^{a} \pm 0{\cdot}794$	$26{\cdot}37^{b} \pm 0{\cdot}595$	NS	*	NS
	G	$27{\cdot}16 \pm 1{\cdot}000$	$26{\cdot}08 \pm 0{\cdot}360$			
$C_{16:1}$	S	$0{\cdot}67 \pm 0{\cdot}045$	$0{\cdot}76 \pm 0{\cdot}071$	NS	*	NS
	G	$0{\cdot}59 \pm 0{\cdot}058$	$0{\cdot}63 \pm 0{\cdot}058$			
$C_{17:0}$	S	$1{\cdot}26 \pm 0{\cdot}086$	$1{\cdot}30 \pm 0{\cdot}096$	NS	NS	NS
	G	$1{\cdot}34 \pm 0{\cdot}092$	$1{\cdot}28 \pm 0{\cdot}053$			
$C_{17:1}$	S	$0{\cdot}39 \pm 0{\cdot}020$	$0{\cdot}44 \pm 0{\cdot}018$	NS	NS	NS
	G	$0{\cdot}52 \pm 0{\cdot}072$	$0{\cdot}42 \pm 0{\cdot}041$			
$C_{18:0}$	S	$18{\cdot}64 \pm 0{\cdot}393$	$18{\cdot}10 \pm 0{\cdot}538$	NS	*	NS
	G	$19{\cdot}78 \pm 0{\cdot}577$	$18{\cdot}57 \pm 0{\cdot}418$			
$C_{18:1}$	S	$24{\cdot}10^{a} \pm 0{\cdot}772$	$29{\cdot}75^{b} \pm 0{\cdot}854$	NS	***	NS
	G	$23{\cdot}69^{a} \pm 1{\cdot}557$	$27{\cdot}78^{b} \pm 0{\cdot}995$			
VA	S	$0{\cdot}58^{A} \pm 0{\cdot}021$	$0{\cdot}58 \pm 0{\cdot}031$	*	NS	*
	G	$0{\cdot}44^{aB} \pm 0{\cdot}032$	$0{\cdot}51^{b} \pm 0{\cdot}037$			
$C_{18:2n6c}$	S	$12{\cdot}68^{a} \pm 0{\cdot}528$	$11{\cdot}09^{b} \pm 0{\cdot}314$	NS	*	NS
	G	$12{\cdot}28 \pm 0{\cdot}825$	$12{\cdot}02 \pm 0{\cdot}520$			
$C_{18:2n6t}$	S	$0{\cdot}16 \pm 0{\cdot}042$	$0{\cdot}18 \pm 0{\cdot}038$	NS	NS	NS
	G	$0{\cdot}14 \pm 0{\cdot}073$	$0{\cdot}19 \pm 0{\cdot}026$			
$C_{18:3n6}$	S	$0{\cdot}35 \pm 0{\cdot}029$	$0{\cdot}31 \pm 0{\cdot}046$	NS	NS	NS
	G	$0{\cdot}31 \pm 0{\cdot}035$	$0{\cdot}30 \pm 0{\cdot}017$			
$C_{18:3n3}$	S	$1{\cdot}54^{aA} \pm 0{\cdot}135$	$1{\cdot}03^{b} \pm 0{\cdot}053$	*	*	**
	G	$0{\cdot}96^{B} \pm 0{\cdot}103$	$1{\cdot}10 \pm 0{\cdot}072$			
$C_{20:0}$	S	$0{\cdot}73^{aA} \pm 0{\cdot}050$	$0{\cdot}55^{b} \pm 0{\cdot}005$	**	***	***
	G	$0{\cdot}54^{B} \pm 0{\cdot}042$	$0{\cdot}53 \pm 0{\cdot}025$			
$C_{20:3n3}$	S	$0{\cdot}44^{a} \pm 0{\cdot}025$	$0{\cdot}39^{b} \pm 0{\cdot}023$	NS	**	NS
	G	$0{\cdot}46^{a} \pm 0{\cdot}048$	$0{\cdot}33^{b} \pm 0{\cdot}024$			
$C_{20:3n6}$	S	$4{\cdot}13^{A} \pm 0{\cdot}188$	$4{\cdot}10^{A} \pm 0{\cdot}154$	***	NS	NS
	G	$5{\cdot}60^{B} \pm 0{\cdot}283$	$5{\cdot}40^{B} \pm 0{\cdot}264$			
$C_{20:5}$	S	$0{\cdot}38^{A} \pm 0{\cdot}060$	$0{\cdot}31^{A} \pm 0{\cdot}032$	***	NS	NS
	G	$0{\cdot}58^{aB} \pm 0{\cdot}043$	$0{\cdot}50^{bB} \pm 0{\cdot}026$			
$C_{23:0}$	S	$0{\cdot}40^{A} \pm 0{\cdot}047$	$0{\cdot}35 \pm 0{\cdot}054$	**	NS	*
	G	$0{\cdot}18^{B} \pm 0{\cdot}039$	$0{\cdot}27 \pm 0{\cdot}023$			
$C_{24:0}$	S	$1{\cdot}54 \pm 0{\cdot}123$	$1{\cdot}50 \pm 0{\cdot}086$	NS	NS	NS
	G	$2{\cdot}06 \pm 0{\cdot}469$	$1{\cdot}63 \pm 0{\cdot}063$			
NEFA	S	$0{\cdot}08^{a} \pm 0{\cdot}008$	$0{\cdot}33^{b} \pm 0{\cdot}040$	NS	***	NS
	G	$0{\cdot}15^{a} \pm 0{\cdot}040$	$0{\cdot}34^{b} \pm 0{\cdot}055$			

Superscripts [1, 2 and 3] as in table 1

VA = trans-11 $C_{18:1}$. This value is not included in the $C_{18:1}$ content

Means with different superscripts with small letters (a,b) in each row for each parameter in sheep and goats (between the dietary treatments) differ significantly ($P \leqslant 0{\cdot}05$)

Means with different superscripts with capital letters (A,B) in each column for each parameter (between sheep and goats in each dietary treatment) differ significantly ($P \leqslant 0{\cdot}05$)

2000; Delbecchi et al. 2001; Ahnadi et al. 2002; Peterson et al. 2003) and goats (Bernard et al. 2005a & b). To our knowledge there is no other study which compares sheep and goats under exactly the same dietary treatments, by quality and quantity, concerning lipogenic (ACC, FAS, LPL and LEP) genes and SCD gene, in their MA. In this study

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the levels on mRNA of SCD in sheep MA were significantly higher compared with those of goats, independently of the fed diet (A or B) and the DIM. This supports the hypothesis of species-specific differences already suggested by Jahreis et al. (1999). The results of mRNA of SCD in MA may partly explain the significantly higher concentrations of *cis-9, trans-11* CLA in sheep milk fat compared with those of goats. In addition sheep milk fat had significantly higher concentrations of VA (the substrate which is used from the Δ^{-9} desaturase in the mammary gland for *cis-9, trans-11* CLA production in milk fat) compared with those of goats, independently from the fed diet (Table 4). These results indicate that not only the mRNA expression of SCD in the mammary gland but and other factors (probably in the rumen) are also responsible for species differences (sheep/goats) in the *cis-9, trans-11* CLA production in milk fat.

It should also pointed out here that even though sheep and goats in this study had different DIM (stage of lactation) this has no effect on their *cis-9, trans-11* CLA milk fat content as it has been shown from previous work with dairy sheep (Tsiplakou et al. 2006b) and cows (Kelsey et al. 2003).

Until now, the observed differences between grazing sheep and grazing goats, in *cis-9, trans-11* CLA milk fat content, had been attributed to differences in grazing behaviour (Nudda et al. 2003; Tsiplakou et al. 2006a). The results of the above study indicated that there are great species differences between sheep and goats in *cis-9, trans-11* CLA milk fat content, with sheep having always higher *cis-9, trans-11* CLA content in their milk fat, which needed to be clarified. To this purpose, the present work shows that the different expression of SCD gene under the same dietary treatment explains in part to what the species differences are possibly due.

The mRNA of SCD could be related with milk *cis-9* $C_{14:1}/C_{14:0}$, *cis-9* $C_{16:1}/C_{16:0}$, *cis-9* $C_{18:1}/C_{18:0}$ and *cis-9, trans-11* $C_{18:2}$ CLA/VA ratios (Table 4) (Bauman et al. 2001), representing a proxy for mammary gland SCD activity. Globally, both mRNA of SCD and Δ^{-9} desaturase ratios were higher in sheep than in goats species with the results being significant for mRNA levels of SCD and *cis-9* $C_{16:1}/C_{16:0}$ independently of the fed diet and for the *cis-9* $C_{14:1}/C_{14:0}$ only for diet B.

Furthermore, in this study was also observed that the Δ^{-9} desaturase activity ratios, expressed by the *cis-9* $C_{14:1}/C_{14:0}$ and *cis-9* $C_{16:1}/C_{16:0}$ ratios, were very low compared with the other two ratios in both animal species independently of the fed diet, because only a small proportion of $C_{14:0}$ and $C_{16:0}$ was desaturated to $C_{14:1}$ and $C_{16:1}$ (Chilliard et al. 2000). A higher Δ^{-9} desaturase activity was estimated in both animal species fed either diet A or diet B, on the basis of the ratio *cis-9* $C_{18:1}/C_{18:0}$ (Table 4). This could be explained by the fact that $C_{18:0}$ is the most preferred substrate of Δ^{-9} desaturase in the mammary gland (Chilliard et al. 2000).

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