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Association of novel SNPs in the candidate genes affecting *caprine* milk fatty acids related to human health

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ABSTRACT

In the present investigation, 618 milk samples of Sirohi breed of goat were collected, and analyzed for conjugated linoleic acid (CLA, C18:2) and other fatty acids. The CLA in studied goat milk samples was 4.87 mg/g of milk fat and C18:2 cis-9, trans-11 contributes 2.9 mg/g of milk fat and trans10 cis12 contributes 0.82 mg/g of milk fat. The saturated fatty acids in the milk accounted for 69.55% and unsaturated fatty acid accounted for 28.50%. The unsaturated fatty acid was constituted by monounsaturated fatty acid (24.57%) and polyunsaturated fatty acids (3.96%.). The major contribution (45.56%) in total fatty acid was of C12:0, C14:0 and C16:0. C18:0 and short chain ones (C4:0, C6:0, C8:0, and C10:0) have a neutral or cholesterol-decreasing effect. The DNA sequence analysis of the genes (DGAT1, SCAP, PPARG, OLR, FABP3 and PRL) in a random panel of 8 Sirohi goats revealed 38 SNPs across the targeted regions. Out of the studied SNPs (38) across these genes, 22 SNPs had significant effect on one or a group of fatty acids including CLA. The genotypes at these loci showed significant differences in the least square means of a particular fatty acid or a group of fatty acids including CLA and its isomers.

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Introduction

Milk is one of the essential parts in human diet rich in all nutritive components. Fatty acids (FA) in milk are becoming more and more important because of their link with certain diseases. Numerous studies, reviewed in Arnould and Soyeurt (2009), have reported that saturated fatty acids (SFA), and especially lauric (C12:0), myristic (C14:0), and palmitic acids (C16:0), have an unfavorable relation with some heart diseases, diabetes, and obesity. In contrast, unsaturated fatty acids (UFA) are reported to have a favorable effect on health, especially on cholesterol levels. However, some short- and medium-chain SFA, such as C6:0 to C10:0, which are well known for their role in the specific goat flavor (C8:0), seem to be of medical interest in humans (mal-absorption syndromes, infant malnutrition, cardiovascular diseases, and non-allergenic properties) (Haenlein, 2004). Caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0) are more abundant in goats; they form 15% to 18% (compared with up to 10% in cow milk) of the total FA (Chilliard et al., 2006; Raynal-Ljutovac et al., 2008). Anti-carcinogenic and anti-atherogenic properties have also been attributed to conjugated linoleic acid (Bauman et al., 2006; Lee et al., 2005; Soyeurt and Gengler, 2008). Moreover, fatty acids are reported to play an important role in the techno-functional properties of cheese making, including organoleptic properties and cheese yield. Milk fat content and composition can be modified by genetic and physiological factors as well as nutritional factors Chilliard et al. (2003). As result, research is currently being carried out on FA content of ruminant milk in different areas of nutrition, physiology and genetics. The present study attempts on identification of SNPs in genes (prolactin, oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1), diacylglycerol O-acyltransferase 1 (DGAT1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGCA), fatty acid binding protein 3 (FABP3) and SREBP cleavage-activating protein (SCAP) linked to fat metabolism and their influence on caprine fat composition to explore its modification to desired level.

Materials and methods

Data collection

The blood and milk samples of Sirohi breed of goat ($N = 618$) were collected during 2010–2012 from All India Coordinated Research Project (AICRP) on goat from different villages of the Udaipur, Ajmer and Chitodgarh districts of Rajasthan. One sample for each animal was collected over 3 seasons viz. summer, winter and spring. Sodium azide was added as preservatives to milk samples so as to analyze them in the lab. A total of 500 µl of 10× solution was added in 100 ml of the milk collected. The samples were stored in refrigerator until further analysis. Blood samples were collected in vaccutainers containing EDTA and were brought to laboratory in cool pack and stored there in refrigerated conditions.

Fatty acid analysis

The method chosen for fatty acid methyl esters (FAME) preparation was from Fallon et al. (2007) with little modifications and has already been presented (Saroha et al., 2014). FAME was prepared directly from milk without prior organic solvent extraction. Identification of FAME was performed from the retention times by using standards of methyl esters. A mixture of the standards of 37 individual FAME (Supelco, Belllefonte, PA) was used to determine response factors. The peak areas in the chromatogram were calculated and normalized using response factors. The individual FA content was expressed as weight percentages (g/100 g of FAME). The conjugated linoleic acid (CLA) was estimated in the milk samples through spectrophotometry. Methyl ester CLA standard of 250 mg/ml was diluted with hexane to a concentration of 25 µg/ml. FAME was diluted 50 times and 230 µl of each sample was loaded in the wells. In addition, wells with hexane only were used as blank. The optical density (OD) was taken at 233 wavelength. Value of CLA in µg/230 µl was calculated from regression equation and then, the concentration of CLA in a given sample was converted to milligram per gram of fat.

Grouping of fatty acids

All FAs were grouped according to their saturation level and chain length. Sum of saturated FA (SFA), sum of short chain saturated FA (C4 to C10, SCFA), sum of medium chain saturated FA (C12 to C15, MCFA) and sum

of long chain saturated FA (C16 to C24, LCFA) were calculated. The remaining 2 groups were the sum of monounsaturated FA (MUFA) and the sum of polyunsaturated FA (PUFA). The index, unsaturated index (UFA multiplied by 100/SFA) was also calculated. The criteria for selecting these FAs depended upon their maximum contribution towards total percentage of FA, with a percentage of more than 3.5% (Arenas et al., 2007) and 3 additional FAs of biological interest (CLA, vaccenic acid (C18:1) and linolenic acid (C18:3)) were also estimated.

DNA extraction and PCR standardization

DNA was extracted from the blood samples of Sirohi goats considered for milk sampling. DNA was extracted using the standard phenol-chloroform method of Sambrook et al. (1989) with little modifications. Polymerase chain reaction (PCR) was performed in a total volume of 25 ml containing about 50–100 ng genomic DNA, 5 pmol/ml of each primer (Sigma Aldrich), 200 mM dNTPs, 1× PCR buffer and 1 U of Taq DNA polymerase (Promega) on advanced Primus 96 PCR machine (PeqLab). The optimization of appropriate annealing temperature with respect to each primer was determined by gradient PCR.

Editing, alignment and identification of SNPs in the candidate genes

After checking the PCR products in agarose gel electrophoresis, PCR products were custom sequenced and the sequence data were analyzed using Chromas (Ver. 1.45, http://www.chromas.html). Chromatogram drawn by data collection software was used to extract the sequence data and was further edited using Chromas 2.13 software to resolve ambiguous bases in the chromatogram. Edited sequences were then used for BLAST analysis to confirm gene identity. Multiple sequence alignments for identification of SNPs were performed with the MegaAlign tool of the LASERGENE software (DNASTAR, Inc, Madison WI, USA). The SNPs observed in heterozygous condition among the Sirohi goats were selected for further genotyping.

SNP genotyping

SNP genotyping was performed by using high-throughput MALDI-TOF mass spectrometry. Primers and probes were designed by using the SpectroDESIGNER software (Sequenom, San Diego). Multiplex PCRs were performed, and unincorporated dNTPs were dephosphorylated by using shrimp alkaline phosphatase (Hoffman-LaRoche, Basel) followed by primer extension. The purified primer extension reaction was spotted onto a 384-element silicon chip (SpectroCHIP, Sequenom) and analyzed in the BrukerBiflex III MALDI-TOF SpectroREADER mass spectrometer (Sequenom), and the resulting spectra processed with SpectroTYPER (Sequenom). Sequence characterization of these genes revealed 38 SNPs across the targeted regions and was genotyped through sequenome in the given sample of goats. The gene and genotypic frequencies of SNPs in studied genes have been presented in Table 1.

Statistical analysis

Statistical analysis was carried out by using the PROC GLM procedure of the SAS (release 9.3) statistical package program. The mathematical model included fixed effects due to parity, year and season of sampling, region (cluster of villages), month of lactation, type of kidding (single or twin) and effects due to genotype. Weight of doe was included as covariate. The variation in 23 dependent variables (15 FA, 7 groups of FA and 1 index) due to factors included in the model was analyzed and least square means were compared using Duncan multiple range test (DMRT).

Results and discussions

Descriptive statistics for CLA and fatty acids

The descriptive statistics for 36 FA$_S$, 7 groups of FA (SFA, MCFA, LCFA, total saturated FA, MUFA, PUFA and UFA) and 1 index (UNSFA/SFA) were studied and shown in Table 2. Analysis of goat milk samples revealed the highest concentration of saturated fatty acids (SFA) out of total milk fatty acids (FA) with an average of 69.55%

Table 1

Gene and genotypic frequencies (GF), Hardy–Weinberg equilibrium (HWE) and polymorphic information content (PIC) across studied genes.

Gene/SNP	Allele	AF	G	GF	PIC	HWE	Gene/SNP	Allele	AF	Genotype	GF	PIC	HWE
DGAT Bt g.7178C>T	C	0.64	C/C	0.41	0.35	HWE	DGAT Bt g.11823A>C	A	0.62	A/A	0.38	0.36	HWE
	T	0.36	C/T	0.46				C	0.38	A/C	0.45		
			T/T	0.12						C/C	0.15		
PRL Bt g.8948A>T	A	0.87	A/A	0.76	0.19	HWE	PRL Bt g.9160T>G	G	0.95	G/G	0.92	0.07	HWE
	T	0.13	A/T	0.22				T	0.05	G/T	0.07		
			T/T	0.01						T/T	0.01		
OLR Bt g.739T>C	C	0.06	C/C	0.04	0.10	HWE	OLR Bt g.2831T>A	A	0.49	A/A	0.23	0.37	HWE
	T	0.94	C/T	0.10				T	0.51	A/T	0.51		
			T/T	0.89						T/T	0.24		
OLR Bt g.2865T>C	C	0.53	C/C	0.28	0.37	HWE	OLR Bt g.2859T>G	G	0.64	G/G	0.40	0.35	HWE
	T	0.47	C/T	0.50				T	0.36	G/T	0.45		
			T/T	0.21						T/T	0.13		
FABP3 Bt g.20539C>G	C	0.99	C/C	0.99	0.00	HWE	FABP3 Bt g.21314A>T	A	0.35	A/A	0.14	0.35	No HWE
	G	0.01	C/G	0.01				T	0.65	A/T	0.41		
										T/T	0.43		
FABP3 Bt g.21321A>T	A	0.23	A/A	0.04	0.29	HWE	FABP3 Bt g.21364G>T	G	0.74	G/G	0.53	0.31	HWE
	T	0.77	A/T	0.36				T	0.26	G/T	0.40		
			T/T	0.58						T/T	0.06		
FABP Bt g.3 21395G>T	G	0.86	G/G	0.74	0.21	HWE	FABP3 Bt g.21485A>G	A	0.25	A/A	0.04	0.31	No HWE
	T	0.14	G/T	0.23				G	0.75	A/G	0.41		
			T/T	0.01						G/G	0.53		
SCAP Bt g.822A>G	A	0.53	A/A	0.31	0.37	No HWE	SCAP Bt g.23453C>T	C	0.48	C/C	0.23	0.37	HWE
	G	0.47	A/G	0.44				T	0.52	C/T	0.50		
			G/G	0.23						T/T	0.26		
SCAP Bt g.23451C>T	C	0.14	C/C	0.01	0.20	HWE	SCAP Bt g.23587C	C	1.0	C/C	1.00	0.0000	
	T	0.86	C/T	0.22									
			T/T	0.75									
SCAP Bt g.38489A>C	A	0.95	A/A	0.95	0.07	No HWE	SCAP Bt g.38572C>G	C	0.58	C/C	0.36	0.37	No HWE
	C	0.05	A/C	0.01				G	0.42	C/G	0.44		
			C/C	0.03						G/G	0.19		

SCAP Bt g.38744C>G	C	0.94	C/C	0.86	0.12	HWE	SCAP Bt g.44173A>G	A	0.21	A/A	0.03	0.28	No HWE
	G	0.06	C/G	0.13				G	0.79	A/G	0.36		
										G/G	0.60		
SCAP Bt g.39466A>G	A	0.47	A/A	0.21	0.37	HWE	SCAP Bt g.39887A>G	A	0.94	A/A	0.87	0.12	HWE
	G	0.53	A/G	0.51				G	0.06	A/G	0.12		
			G/G	0.27						G/G	0.01		
SCAP Bt g.38490A>C	A	0.95	A/A	0.95	0.07	No HWE	SCAP Bt g.41251A>T	A	0.32	A/A	0.11	0.34	HWE
	C	0.05	A/C	0.01				T	0.68	A/T	0.40		
			C/C	0.03						T/T	0.47		
SCAP Bt g.41348C>G	C	0.23	C/C	0.07	0.30	No HWE	SCAP Bt g.41373A>G	A	0.43	A/A	0.19	0.36	HWE
	G	0.77	C/G	0.31				G	0.57	A/G	0.46		
			G/G	0.60						G/G	0.34		
SCAP Bt g.g.43891G>A	A	0.86	A/A	0.73	0.20	HWE	SCAP Bt g.43998G>A	A	0.29	A/A	0.09	0.32	HWE
	G	0.14	A/G	0.25				G	0.71	A/G	0.40		
			G/G	0.01						G/G	0.50		
PPARGCA Bt g.85956C>A	A	0.06	A/C	0.11	0.10	HWE	PPARG Bt g.85964G>T	G	0.34	G/G	0.10	0.35	HWE
	C	0.94	C/C	0.88				T	0.66	G/T	0.48		
										T/T	0.40		
PPARGCA Bt g.86054A>G	A	0.31	A/A	0.11	0.33	No HWE	PPARGCA Bt g.86060A>T	A	0.24	A/A	0.06	0.29	HWE
	G	0.69	A/G	0.39				T	0.76	A/T	0.33		
			G/G	0.49						T/T	0.60		
PPARGCA Bt g.T>G 86127	G	0.58	G/G	0.34	0.36	HWE	PPARG Bt g.86169G>T	G	0.54	G/G	0.27	0.37	No HWE
	T	0.42	G/T	0.48				T	0.46	G/T	0.51		
			T/T	0.17						T/T	0.20		
SCAP Bt g.596A>C	A	0.74	A/A	0.56	0.30	HWE	FABP3 Bt g.21364G>T	G	0.74	G/G	0.53	0.31	HWE
	C	0.26	A/C	0.37				T	0.26	G/T	0.40		
			C/C	0.06						T/T	0.06		

Bt = *Bos taurus*.

Table 2
Milk composition and percent contribution of each fatty acid.[a]

Fatty acid	Nomenclature	Mean	Minimum	Max	SD
CLA[b]	c18:2	4.873	0.393	16.724	2.878
cis9tran11	C18:2 cis 9 trans 11	2.943	0.0063	7.875	1.433
trans10 cis12	C18:2 trans 10 cis 12	0.823	0.021	3.592	0.824
Butyric acid	c4:0	1.349	0.020	7.272	1.230
Caproic acid	c6:0	2.611	0.415	15.73	2.033
Caprylic acid	c8:0	3.660	0.463	9.722	1.612
Capric acid	c10:0	6.750	0.266	20.953	4.531
Undecanoic acid	c11:0	1.744	0.159	17.897	5.429
Lauric acid	c12:0	6.825	1.772	20.045	4.278
Short chain fatty acid	SCFA	13.461	2.239	33.631	6.818
Tridecanoic acid	c13:0	0.5886	0.136	14.386	3.276
Myristic acid	c14:0	11.770	0.315	24.881	3.899
Myristoleic acid	c14:1	1.353	0.113	15.465	2.600
Pentadecanoic acid	c15:0	1.667	0.0585	24.902	4.005
cis10-pentadecenoic acid	c15:1	0.494	0.121	11.133	1.278
Palmitic acid	c16:0	26.991	1.097	41.707	5.730
Palmitoleic acid	c16:1	2.731	0.206	19.129	2.379
Medium chain fatty acid	MCFA	20.056	7.470	45.270	6.096
Hepiadecanoic acid	c17:0	0.757	0.196	9.679	1.533
cis-10-heptadecenoic	c17:1	0.832	0.0567	18.287	2.233
Stearic acid	c18:0	7.665	0.3752	21.297	4.075
Elaidic acid	c18:1n9t	1.497	0.240	19.946	3.087
Oleic acid	c18:1n9c	19.088	0.783	31.947	6.222
Linolelaidic acid	c18:2n6t	0.735	0.141	6.778	1.526
Gamma linolenic acid	c18:3n6	1.682	0.0754	7.948	1.089
Linoleic acid	c18:2n6c	2.423	0.0257	15.063	3.015
Arachidic acid	c20:0	0.700	0.0957	13.892	2.532
cis-11-eicosenoic acid	c20:1	0.735	0.088	17.937	2.131
Linolenic acid	c18:3 n3	0.255	0.053	4.243	0.808
Heneicosanoic acid	c21:0	0.371	0.055	11.081	1.841
cis11,14 eicosadienoic acid	c20:2	0.215	0.102	2.887	0.878
Long chain fatty acid	LCFA	35.089	4.774	51.228	5.313
Saturated fatty acid	SFA TOTAL	69.595	43.263	88.053	5.446
Mono unsaturated fatty acid	MUFA	24.572	4.790	39.408	5.384
Polyunsaturated fatty acid	PUFA	3.966	0.592	18.309	3.593
Unsaturated fatty acid	USFA	28.502	10.449	45.741	5.236
Unsaturated index	USFA[a]100/SFA	41.595	12.575	84.303	10.196

[a] Fatty acids are measured as g per 100 g of FAME
[b] CLA was measured as mg per g of milk fat.

ranging from 43.26 to 88.05. Within saturated fatty acid, the major contribution was palmitic (C16:0) 26.99% followed by myristic (C14:0) 11.77%, stearic (C18:0) 7.66% and capric (C10:0) 6.75%, respectively. The major contribution (45.56%) in total FAs was C12:0, C14:0 and C16:0, and C18:0. The short chain FAs (C4:0, C6:0, C8:0, and C10:0) have a neutral or cholesterol-decreasing effect and contributed 14.37% to the pool of FAs. Unsaturated fatty acids (UFA) are extremely important for human health. The highest monounsaturated fatty acid (MUFA) levels were those of oleic acid (C18:1 cis-9) — 19.08%. The average content of trans-isomers of C18:1 varied between 0.240% and 19.94%. Polyunsaturated fatty acid (PUFA) contributed 3.96%. The total sum of CLA in studied goat milk was 4.87 mg/g of milk fat and the biologically active isomers C18:2 cis-9 trans-11 and C18:2 trans-10 cis-12 contributed 2.9 and 0.82 mg/g of fat respectively. The goat specific FAs (c6:0, c8:0 and c10:0) contribute 13% to the total FAs.

Gene and genotypic frequencies

Gene and genotypic frequencies, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) across studied genes have been presented in Table 1. All the heterozygous loci (38) identified across 6 genes could be assayed for their genotyping in the given sample of Sirohi breed of goat. All the loci were in

Hardy–Weinberg equilibrium (HWE) except 12 loci (10 in SCAP and 2 in FABP3) across different genes. Most of these loci had polymorphic information content value of more than 0.30. The wide range of PIC values (0.0–0.375) indicated very low to high polymorphism across the loci. Linkage disequilibrium across many studied loci particularly in SCAP gene (where all the loci were in LD) makes them ideal markers for their association with different fatty acids including CLA and its isomers. Moderate to high PIC values of the loci which are found to be in LD make them suitable candidates for association studies.

Association of genotypes/haplotypes with CLA, its isomers and other fatty acids

Significant association of different genotypes across studied genes with CLA and other fatty acids has been presented in Table 3. The SNPs in LD were taken up for further analysis of genotype association with 36 fatty acids including the conjugated linoleic fatty acid (CLA), and its two important isomers as well as unsaturation index under study. The least squares means of important traits viz CLA (C18:2) and its two health related isomers (cis9 trans 11 and cis 10 trans 12), and other important fatty acids have been presented in Table 3. Genes having significant effect on these traits are discussed below.

Fatty acid binding protein 3 (FABP3)

Milk components originating from blood plasma substrates are synthesized in epithelial cells of the mammary gland. Milk lipids are synthesized from fatty acids which bind to specific proteins — FABPs (fatty acid binding proteins). FABPs are a family of small cytoplasmic proteins; nine members of the family have been identified so far (FABP1–FABP9) Chmurzyńska (2006). Their main roles include fatty acid uptake, transport and metabolism. FABPs can modulate the fatty acid concentration in cells and therefore they affect different cellular processes, especially lipid metabolism. FABP3 and FABP4 are present in tissues with a high demand for fatty acids, such as heart muscle, skeletal muscles, lactating mammary gland, liver or adipose tissue (Roy et al., 2003). FABP3 gene was mapped to bovine chromosome 2 (Calvo et al., 2004), where QTLs affecting milk fat yield and content were described (Khatkar et al., 2004). In this study, the different loci at FABP3 gene contributed 1.2% to 2.0% to the total explained variance of different fatty acids by the fitted model. FABP3 g.21395G>T significantly influenced butyric (C4:0) and arachidic acid (C20:0) whereas g.20539C>G significantly affected butyric acid (C4:0). CG does were associated with five times more C4:0 (5.89%) in their milk compared to CC and GG does whereas TT genotypes (1.21%) yielded more than three times arachidic FA as compared to GT/GG genotypes. FABP3 g.21321A>T showed significant effect on total saturated fatty acid as well as monounsaturated fatty acid (MUFA), total unsaturated fatty acid and unsaturation index. Allele A was associated with higher (1.5%) saturated fatty acids and lower (8%) unsaturated fatty acids particularly MUFA and unsaturation index while the reverse was true with allele T. SNP g.21364G>T influenced CLA cis 9 trans 11. Heterozygous GT does were associated with 41% more CLA cis 9 trans 11 compared with the homozygous TT does. Associations between SNPs in these genes and caprine milk fat composition have not been reported so far. However, SNPs within FABP3 have been reported to influence fat and protein percentage in cattle (Kulig et al., 2010). The relationship between FABP3 polymorphism and milk C18:2 cis 9 trans 11 content is of particular interest. Milk C18:2 cis 9 trans 11 is characterized by multi-fold individual variation. Therefore, an important role of the genetic background of does could be hypothesized. Moreover, 8 fold difference in milk C18:2 cis 9 trans 11 of cows had also been reported in literature Bumann et al. (2000).

Prolactin (PRL)

In the lactating mammary gland, prolactin (PRL) stimulates the synthesis of lactose as well as fatty acid uptake, lipogenesis, and triacylglycerol synthesis. Here in this study, the locus explained 1.03% of the total explained variance of the fatty acids defined by this gene. Associations between bovine PRL receptor (PRLR) genotype and fat yield have earlier been reported (Viitala et al., 2006), which illustrates the role of PRL in conveying lipids toward the udder as well as in stimulating their local synthesis during lactation. SNP g.8948A>T had significant effect on total saturated fatty acid particularly long chain saturated FA (arachidic acid). The locus also influenced MUFA. The allele T favors the saturation while allele A favors the unsaturation by more than 8% in homozygous condition. The association analysis with milk composition traits in a Murciano-Granadina goat population also revealed highly suggestive effects on palmitoleic acid content,

Table 3
Least square means of important fatty acids by genotypes.

Gene/genotype	CLA[a]	cisn	cisot	sat	ssfa	mfa	lfa	mufa	pufa	ufa	inu
FABP3 Bt g.21321A>T				**				**		**	**
AA	3.14 ± 0.69	1.66 ± 0.4	0.59 ± 0.19	69.9b ± 1.27	13.36 ± 1.39	23.98 ± 1.5	30.71 ± 2.0	24.6a ± 1.35	4.5 ± 0.74	29.06a ± 1.4	42.18a ± 2.8
TA	3.52 ± 0.50	1.77 ± 0.3	0.60 ± 0.14	70.1b ± 0.91	13.42 ± 1.00	23.31 ± 1.1	32.46 ± 1.4	25.5b ± 0.97	3.8 ± 0.53	29.33a ± 0.9	42.58a ± 2.0
TT	3.92 ± 0.50	1.88 ± 0.3	0.64 ± 0.14	68.8a ± 0.91	13.19 ± 1.00	23.23 ± 1.1	31.41 ± 1.4	26.8c ± 0.97	4.0 ± 0.53	30.8b ± 0.99	45.4b ± 1.99
PRl Bt g.8948A>T				*				*	*		
AA	3.74 ± 0.49	1.84 ± 0.2	0.63 ± 0.13	69.2a ± 0.90	13.21 ± 0.98	23.41 ± 1.0	31.5a ± 1.4	26.4c ± 0.96	3.9 ± 0.52	30.30 ± 0.98	44.52 ± 1.96
AT	3.66 ± 0.53	1.79 ± 0.3	0.59 ± 0.14	70.34b ± 0.9	13.67 ± 1.05	22.90 ± 1.1	33.14b ± 1.	25.23b ± 1.0	4.06 ± 0.5	29.29 ± 1.05	42.40 ± 2.11
TT	3.28 ± 1.27	1.83 ± 0.7	0.36 ± 0.35	71.51c ± 2.3	10.12 ± 2.53	20.91 ± 2.8	37.26c ± 3.	24.34a ± 2.4	4.22 ± 1.3	28.57 ± 2.54	40.18 ± 5.08
FABP3 Bt g.20539C>G	**	*	*								
CC	3.73a ± 0.4	1.83a ± 0.	0.62a ± 0.1	69.49 ± 0.89	13.30 ± 0.97	23.27 ± 1.0	31.92 ± 1.4	26.12 ± 0.95	3.93 ± 0.5	30.05 ± 0.98	44.00 ± 1.96
CG	9.87b ± 2.6	4.4b ± 1.0	2.06b ± 0.7	73.04 ± 4.92	13.85 ± 5.34	16.30 ± 5.9	42.32 ± 7.7	25.93 ± 5.25	1.71 ± 2.8	27.64 ± 5.37	38.16 ± 10.7
OLR Bt g.739T>C			*								
CC	5.43 ± 1.59	3.18 ± 0.8	1.88b ± 0.4	72.59 ± 2.91	14.87 ± 3.16	26.07 ± 3.5	31.52 ± 4.5	18.56a ± 3.1	5.37 ± 1.6	23.96 ± 3.17	33.17 ± 6.35
TC	3.39 ± 0.59	1.87 ± 0.3	0.58a ± 0.2	69.69 ± 1.08	12.49 ± 1.17	24.17 ± 1.3	31.81 ± 1.7	26.32b ± 1.1	3.61 ± 0.6	29.94 ± 1.17	43.75 ± 3.35
TT	3.73 ± 0.49	1.81 ± 0.2	0.61a ± 0.1	69.44 ± 0.90	13.36 ± 0.97	23.19 ± 1.0	31.91 ± 1.4	26.15b ± 0.9	3.96 ± 0.5	30.11 ± 0.97	44.10 ± 1.96
SCAP Bt g.596A>C								*			
AA	3.59 ± 0.49	1.79 ± 0.3	0.59 ± 0.13	69.64 ± 0.91	13.48 ± 0.99	23.05 ± 1.1	32.05 ± 1.4	25.98b ± 0.9	3.86 ± 0.5	29.84 ± 0.99	43.63 ± 1.99
CA	3.92 ± 0.51	1.92 ± 0.2	0.66 ± 0.14	69.09 ± 0.93	13.10 ± 1.01	23.67 ± 1.1	31.46 ± 1.4	26.57b ± 0.9	3.97 ± 0.5	30.55 ± 1.01	44.93 ± 2.03
CC	3.66 ± 0.64	1.65 ± 0.3	0.70 ± 0.18	70.43 ± 1.17	12.76 ± 1.28	23.20 ± 1.4	33.39 ± 1.8	24.44a ± 1.2	4.63 ± 0.7	29.08 ± 1.28	41.83 ± 2.57
FABP3 Bt g.21364G>T		*									
GG	3.76 ± 0.50	1.83b ± 0.	0.62 ± 0.14	69.61 ± 0.92	13.27 ± 1.00	23.12 ± 1.1	32.31 ± 1.4	26.11 ± 0.99	3.86 ± 0.5	29.97 ± 1.01	43.82 ± 2.02
GT	3.96 ± 0.51	2.08c ± 0.	0.66 ± 0.14	69.13 ± 0.95	13.57 ± 1.03	23.15 ± 1.1	31.43 ± 1.5	26.29 ± 1.01	3.91 ± 0.5	30.21 ± 1.04	44.44 ± 2.08
TT	3.27 ± 0.61	1.47a ± 0.	0.56 ± 0.17	69.63 ± 1.13	13.00 ± 1.22	23.89 ± 1.3	31.54 ± 1.7	26.88 ± 1.20	4.17 ± 0.6	30.06 ± 1.23	43.88 ± 2.47
PPARG Bt g.86169G>T								*		*	
GG	3.56 ± 0.51	1.88 ± 0.2	0.69 ± 0.14	70.05 ± 0.93	13.84 ± 1.01	23.62 ± 1.1	31.56 ± 1.5	25.28a ± 0.9	4.02 ± 0.5	29.31a ± 0.1	42.57 ± 2.04

TG	3.74 ± 0.50	1.78 ± 0.2	0.56 ± 0.14	69.16 ± 0.92	13.17 ± 1.00	23.30 ± 1.1	31.79 ± 1.4	26.60b ± 0.9	3.94 ± 0.5	30.54b ± 1.0	44.94 ± 2.02
TT	3.98 ± 0.53	1.82 ± 0.3	0.62 ± 0.14	69.01 ± 0.97	12.54 ± 1.06	22.60 ± 1.1	32.77 ± 1.5	26.76b ± 1.0	3.79 ± 0.5	30.56b ± 1.1	44.96 ± 2.13
SCAP Bt g.39887A>G											
AA	3.76 ± 0.49	1.84 ± 0.3	0.63 ± 0.13	69.53 ± 0.90	13.35a ± 0.9	23.36 ± 1.0	31.80 ± 1.4	26.08 ± 0.96	3.96 ± 0.5	30.05 ± 0.98	43.98 ± 1.96
GA	3.32 ± 0.57	1.77 ± 0.3	0.57 ± 0.16	69.05 ± 1.06	12.92a ± 1.1	22.63 ± 10	32.74 ± 1.6	26.40 ± 1.13	3.75 ± 0.6	30.15 ± 1.15	44.29 ± 2.31
GG	4.82 ± 1.92	2.45 ± 1.0	1.57 ± 0.54	69.29 ± 3.53	21.98b ± 3.8	22.63 ± 1.2	25.27 ± 5.5	23.55 ± 3.76	7.42 ± 2.0	30.98 ± 3.85	45.85 ± 7.70
	*		*		**						
SCAP Bt g.43891G>A											
AA	3.83b ± 0.5	1.87 ± 0.2	0.66b ± 0.1	69.33 ± 0.90	13.17b ± 0.9	23.51b ± 1.	31.63 ± 1.4	26.16 ± 0.96	4.06 ± 0.5	30.22 ± 0.98	44.31 ± 1.97
AG	3.17a ± 0.5	1.74 ± 0.3	0.51a ± 0.1	69.83 ± 0.98	13.55b ± 1.0	22.53a ± 1.	13.84 ± 1.5	25.92 ± 1.04	3.67 ± 0.5	29.59 ± 1.06	43.21 ± 2.13
GG	4.96c ± 1.27	3.00 ± 0.66	1.07c ± 0.3	69.95 ± 2.34	7.21a ± 2.53	29.20c ± 2.	32.03 ± 3.7	25.91 ± 2.50	4.03 ± 1.3	29.95 ± 2.55	43.43 ± 5.12
								*		*	*
SCAP Bt g.39466A>G											
AA	3.95 ± 0.53	1.83 ± 0.27	0.63 ± 0.14	68.99 ± 0.97	12.51 ± 1.06	22.62 ± 1.17	32.78 ± 1.53	26.80b ± 1.0	3.77 ± 0.5	30.58b ± 1.1	44.99b ± 2.1
GA	3.73 ± 0.50	1.78 ± 0.26	0.55 ± 0.14	69.16 ± 0.92	13.16 ± 1.00	23.30 ± 1.12	31.79 ± 1.45	26.61b ± 0.9	3.93 ± 0.5	30.55b ± 1.0	44.95b ± 2.0
GG	3.57 ± 0.51	1.87 ± 0.26	0.68 ± 0.14	70.06 ± 0.93	13.86 ± 1.01	23.61 ± 1.13	31.55 ± 1.47	25.26a ± 0.9	4.03 ± 0.5	29.29a ± 1.0	42.55a ± 2.0
								*			
SCAP Bt g.23451C>T											
CC	3.84 ± 0.53	1.77 ± 0.27	0.59 ± 0.14	68.90 ± 0.97	12.78 ± 1.05	22.73 ± 1.17	32.34 ± 1.52	26.81b ± 1.0	3.84 ± 0.5	30.66 ± 1.05	45.18 ± 2.11
CT	3.78 ± 0.50	1.81 ± 0.26	0.58 ± 0.14	69.17 ± 0.92	13.02 ± 1.00	23.02 ± 1.11	32.16 ± 1.45	26.52b ± 0.9	3.83 ± 0.5	30.35 ± 1.01	44.62 ± 2.02
TT	3.57 ± 0.51	1.87 ± 0.26	0.68 ± 0.14	70.12 ± 0.93	13.88 ± 1.01	23.87 ± 1.13	31.38 ± 1.47	25.31a ± 0.9	4.11 ± 0.5	29.43 ± 1.02	42.74 ± 2.04
								*			
OLR Bt g.2865T>G											
GG	3.81 ± 0.51	1.93 ± 0.26	0.66 ± 0.14	70.24b ± 0.9	13.51 ± 1.02	23.23 ± 1.12	32.52 ± 1.46	25.27a ± 0.9	4.00 ± 0.5	29.28a ± 1.0	42.51a ± 2.0
GT	3.65 ± 0.50	1.75 ± 0.26	0.59 ± 0.14	69.07a ± 0.9	13.27 ± 0.99	23.56 ± 1.09	31.29 ± 1.42	26.57b ± 0.9	3.99 ± 0.5	30.56b ± 0.9	44.96b ± 1.9
TT	3.83 ± 0.56	1.97 ± 0.29	0.65 ± 0.15	69.34a ± 1.0	12.73 ± 1.13	22.19 ± 1.24	33.20 ± 1.62	26.23b ± 1.1	3.51 ± 0.6	29.75ab ± 1.	43.48ab ± 2.
								*		*	*
PPARG Bt g.85964G>T											
GG	3.61 ± 0.58	1.83 ± 0.30	0.73 ± 0.16	69.15 ± 1.07	11.55a ± 1.1	22.96 ± 1.30	33.54 ± 1.68	26.47 ± 1.15	3.89 ± 0.6	30.37 ± 1.17	44.37 ± 2.35
GT	4.02 ± 0.50	1.89 ± 0.26	0.61 ± 0.14	69.15 ± 0.93	13.29b ± 1.0	23.31 ± 1.12	31.69 ± 1.45	26.38 ± 0.99	4.05 ± 0.5	30.44 ± 1.01	44.81 ± 2.03
TT	3.50 ± 0.50	1.81 ± 0.26	0.61 ± 0.14	69.79 ± 0.92	13.70b ± 0.9	23.41 ± 1.11	31.57 ± 1.44	25.79 ± 0.98	3.91 ± 0.5	29.71 ± 1.00	43.36 ± 2.01

CLA: conjugated linoleic fatty acid, cisn: CLA cis 9 trans 11, cisot: CLA cis10 trans 12, sat: total saturated FA, ssfa: total short chain FA, mfa: medium chain saturated FA, lfa: long chain saturated FA, mufa: mono unsaturated FA, pufa: polyunsaturated FA, ufa: unsaturated FA, inu: unsaturation index. CLA measured in mg/g fat, other FAs measured as g/100 g FAME.

Bt: *Bos taurus*, * $p \leq 0.05$ and ** $p \leq 0.01$.

whereas suggestive effects were detected for other fatty acids, such as palmitic and linoleic (Zidi et al., 2010). The present results are consistent with the pleiotropic effects of PRL on mammary gland lipid metabolism and milk composition.

Oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1)

OLR1 is involved in fatty acid transport and binds and degrades the oxidized form of low density lipoprotein. OLR1 locus contributed 1.10% to 1.87% to the total variance explained by the model in the traits influenced by the SNPs at this locus. SNP g.2865T>G significantly affected saturated and unsaturated FA content in the goat milk. The homozygous GG does produce more (>1%) saturated FA in their milk than heterozygous/ homozygous GT/TT does. This locus also affected lauric (C12:0) FA content in the milk with GG geno-types (7.21%) yielding about 1% more lauric FA. Allele T favors unsaturation (3.8%) particularly for the MUFA and G favors saturation of FA as revealed by saturated FA content and unsaturation index. The locus g.2859C>T had affected lauric (C12:0) and myristic (C14:0) FA content in the given samples. The homozygous TT individuals were characterized by 1% more C12:0 whereas heterozygous CT does were characterized by 1% more of C14:0 compared with alternative genotypes at this locus. The locus g.2831A>T showed significant effect on caprylic (C8:0), a goat specific FA and oleic FA (c18:1n9c) with TT genotypes yielding almost 1% more of these two FAs. The results revealed pleiotropic effect of OLR1 gene on caprine milk fat composition. SNPs within the bovine OLR1 gene have also been reported to have a significant effect on milk-fat percentage and on milk-fat composition (Khatib et al., 2006; Schennink et al., 2009).

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGCA)

PPARG is a critical transcriptional regulator of genes controlling energetic metabolism, adipogenesis and maintenance of the differentiated state (Memisoglu et al., 2003; Rosen and MacDougal, 2006; Xu et al., 1999). In the present study, PPARGCA locus characterized caprine milk fatty acids by 1.0% to 1.5% in terms of the explained variance. The locus g.86169G>T had significant effect on MUFA and total unsaturated FA content in the goat milk. TT does were characterized by significantly higher content of MUFA (5.8%) and total unsaturated FA (4.2%) in milk as compared to GG does. This locus also influenced oleic FA (c18:1n9c) with TT genotypes having 18.58% of oleic FAs that were found to be 1% superior to other genotypes. In contrast to this locus, SNP g.85964G>T affected short chain saturated FA. The homozygous GG individuals were characterized by 15.7% lesser content of short chain saturated FA as compared to TT individuals. No domi-nance effect was also observed at the locus. However, the locus also affected long chain saturated FA (arachidic, C:20). The homozygous GG genotypes (0.71%) were associated with higher content (2.8%) of C:20 compared to other genotypes. The locus g.86060A>T was associated with the caproic acid and the geno-type AA (2.82%) was superior to the TA (2.51%) and TT (2.38%). Previously, SNPs within bovine PPARGCA gene have also been reported to have a significant effect on milk-fat percentage and on milk-fat composition (Schennink et al., 2009). Also consistent with the current results, Oh et al. (2011) found an exonic SNP of PPARG associated with both SFA and MUFA in Korean cattle.

SREBP cleavage-activating protein (SCAP)

Genes in the sterol regulatory element-binding protein-1 (SREBP1) pathway play a central role in the regulation of milk fat synthesis, especially the de novo synthesis of saturated fatty acids. In the present study, we discovered SNP in SREBP1 genes to identify genetic markers that can be used for genetic and met-abolically directed selection in goat. We identified SNPs in this pathway influencing short chain saturated FA, MUFA, unsaturated fatty acid content and unsaturation index by 0.17% to 1.90% of the explained variance in these traits. SNP g.596A>C showed significant effect on MUFA content in the milk. Homozygous AA does had higher (6.3%) content of MUFA in their milk as compared to CC does. Moreover, there was no dominance effect of the locus on MUFA. On other hand, g.39887A>G had affected total short chain saturated FA content in the milk. GG individuals had higher (about 64%) content of short chain saturated FA as compared to AA/AG individuals. This locus also influenced linoleic acid (c18:2n6c) and genotype GG (6.43%) was found to be superior to AA (1.76%) and AG (1.46%) genotypes. The SNP g.41373A>G significantly influenced the oleic

FA with genotype AG (18%) being associated with 1% more of this FA than AA and GG genotypes. The locus g.43891G>A revealed significant association with CLA and its isomer cis 10 trans 12, total short chain saturated FA, capric FA (C10:0) and medium chain FA in the goat milk. Allele G in homozygous condition favors higher content of CLA (29.5%) and its isomer (62%), and medium chain FA (24%) but leads to significant reduction in short chain FA. This locus also influenced the capric FA and the genotype AG (7.40%) was found to be superior to the AA (6.92%) and GG (2.33%) genotypes. The locus g.43998A>G influenced the arachidic FA and the genotype GG (0.41%) was found to be superior to the AG (0.14%) and AA (0.10%) genotypes. SNPs g.39466A>G showed a significant effect on MUFA, unsaturated fatty acid content and unsaturation index. AA genotypes had higher content of MUFA (6%), unsaturated fatty acid content (4.4%) and unsaturation index (5.7%) compared to GG genotype. The locus also affected oleic FA and genotype AA (18.65%) was found to be superior to the AG (17.22%) and GG (16.37%) genotypes. Similarly allele C favors higher content of MUFA at g.23451C>T. The SNP g.23451C>T also affected lauric and oleic FA. However, the differences among alternative genotypes were not significant enough. The SNP g.23490C>G affected gamma linoleic FA and the genotype CC (1.62%) was found to be superior to the GC (0.90). The SNP g.23453C>T had significant association with arachidic acid. TC genotype was found to be superior to TT and CC. SNP g.39758A>T also had significant association with myristic acid. TT (12.44%) genotype was found to be superior to AA (12.20%) and TA (10.49%). Consistent with these functions, several polymorphisms in SREBF1 were previously associated with meat FA composition in cattle (Hoashi et al., 2007) and intramuscular fat (Chen et al., 2008) and leg weight (Renaville et al., 2010) in pigs, and SNPs in SCAP were correlated with lean percentage, back-fat thickness, fat color and salting losses in pigs (Renaville et al., 2010). It was interesting to note that no SNP within bovine SCAP gene was associated with milk-fat composition (Conte et al., 2010) which might have probably been due to a small number of animals genotyped against the SNPs. The results revealed that SREBP-1 has a central role in defining the various fatty acids in goat milk. The markers in this gene could be direct causal factors or linked to fatty acids quantitative trait loci (QTLs).

Association of haplotypes with CLA, its isomers and other FAs

Six haplotypes (CAAG, CATA, CATG, CTAA, CTTA, and CTTG) were observed in FABP3 gene and the effect of haplotypes on CLA was significant ($p < 0.06$). The haplotype CTTA (4.47 mg/g of fat) was superior to haplotype CTAA (3.35 mg/g of fat). All 26 SNPs observed in the SCAP gene were found to be in LD with each other, but haplotype and association analyses were not carried out because there were a very large number of haplotypes ($N = 351$) with very small sample size (three or less data per haplotype).

Conclusions

In the present study, 38 novel SNPs have been identified in six key genes involved in lipid metabolism of caprine milk. The identification of SNPs in genes responsible for the variation of milk fat composition described herein provides useful information that can be combined with breeding programs to tailor fatty acid content in caprine milk. The variability in CLA and other FA content revealed the scope for suitable breeding and management strategies to improve their concentration in goat milk and thereby scope for value addition to goat milk and milk products.

Acknowledgments

References

Arenas, R., Barbosa, E., Prieto, B., San Primitivo, F., Fresno, J.M., De La Fuente, L.F., 2007. Variaciones temporales del contenido de ácidos grasos de leche de oveja. IV Jornadas Ibéricas de razas autóctonas y sus productos tradicionales. Publ. Junta de Andalucía, Sevilla, Spain, pp. 201–204.

Arnould, V.M.R., Soyeurt, H., 2009. Genetic variability of milk fatty acids. J. Appl. Genet. 50 (1), 29–39.

Bauman, D.E., Mather, I.H.R., Wall, J., Lock, A.L., 2006. Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89, 1235–1243.

Bumann, C.A., Ribon, V., Kanzaki, M., Thurmond, D.C., Mora, S., Shigematsu, S., Bickel, P.E., Pessin, J.E., Saltiel, A.R., 2000. CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 407, 202–207.

Calvo, J.H., Marcos, S., Jurado, J.J., Serrano, M., 2004. Association of the heart fatty acid-binding protein (FABP3) gene with milk traits in Manchega breeds sheep. Anim. Genet. 35, 347–349.

Chen, S.N., Cilingiroglu, M., Todd, J., 2008. Candidate genetic analysis of plasma high-density lipoprotein-cholesterol and severity of coronary atherosclerosis. BMC Med. Genet. 10 (111).

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

Chilliard, Y., Ferlay, A., Mansbridge, R.M., Doreau, M., 2006. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. INRA. Ann. Zootech. 49, 181–205.

Chmurzyńska, A., 2006. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J. Appl. Genet. 47, 39–48.

Conte, G., Mele, M., Chessa, S., Castiglioni, B., Serra, A., Pagnacco, G., Secchiari, P., 2010. Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle. J. Dairy Sci. 93, 753–763.

Fallon, J.V.O., Busboom, J.R., Nelson, M.L., Gaskins, C.T., 2007. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues oils, and feed stuffs. J. Anim. Sci. 85, 1511–1521.

Haenlein, G.F.W., 2004. Goat milk in human nutrition. Small Rumin. Res. 51, 154–163.

Hoashi, S., Ashida, N., Ohsaki, H., Utsugi, T., Sasazaki, S., Taniguchi, M., Oyama, K., Mukai, F., Mannen, H., 2007. Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in Japanese Black cattle. Mamm. Genome 18, 880–886.

Khatib, H., Leonard, S.D., Schutzkus, V., Luo, W., Chang, Y.M., 2006. Association of the OLR1 gene with milk composition in Holstein dairy cattle. J. Dairy Sci. 89 (5), 1753–1760.

Khatkar, M.S., Thomson, P.C., Tammen, I., Raadsma, H.W., 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. 36, 163–190.

Kulig, H., Kowalewska-Łuczak, I., Kmieć, M., Wojdak-Maksymiec, K., 2010. ANXA9, SLC27A3, FABP3 and FABP4 single nucleotide polymorphisms in relation to milk production traits in Jersey cows Czech J. Anim. Sci. 55, 463–467.

Lee, K.W., Lee, H.J., Cho, H.Y., Kim, Y.J., 2005. Role of the conjugated linoleic acid in the prevention of cancer. Crit. Rev. Food Sci. Nutr. 45 (2), 135–144.

Memisoglu, A., Hu, F.B., Hankinson, S.E., Manson, J.E., De Vivo, I., Willett, W.C., 2003. Interaction between a peroxisome proliferator-activated receptor γ gene polymorphism and dietary fat intake in relation to body mass. Hum. Mol. Genet. 12, 2923–2929.

Oh, D., Lee, Y., Lee, C., Chung, E., Yeo, J., 2011. Association of bovine fatty acid composition with missense nucleotide polymorphism in exon7 of peroxisome proliferator activated receptor gamma gene. Anim. Genet. 43, 474.

Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I., Chilliard, Y., 2008. Composition of goat and sheep milk products. Small Rumin. Res. 79, 57–72.

Renaville, B., Glenn, K.L., Mote, B.E., Fan, B., Stalder, K.J., Rothschild, M.F., 2010. SREBP pathway genes as candidate markers in country ham production. Ital. J. Anim. Sci. 9, 7.

Rosen, E.D., MacDougal, O.A., 2006. Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7, 885–896.

Roy, R., Calvo, J.H., Hayes, H., Rodellar, C., Eggen, A., 2003. Fine mapping of the bovine heart fatty acid-binding protein gene (FABP3) to BTA2q45 by fluorescence in situ hybridization and radiation hybrid mapping. Anim. Genet. 34, 466–467.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning, A Laboratory Manual. Cold Spring Harbour Lab. Press, Cold Spring Harbour, N.Y.

Saroha, V., Kumar, D., Sharma, A., Jayakumar, S., Tyagi, A.K., Nagda, R.K., Dixit, S.P., 2014. Quantitative analysis of fatty acid in Indian goat milk and its comparison with other livestock. J. Livest. Sci. 5, 1–8.

Schennink, A., Bovenhuis, H., Leon- Kloosterziel, K.M., Van Arendonk, J.A., Visker, M.H., 2009. Effect of polymorphisms in the FASN, OLR1, PPARGCiA, PRL and STST5A genes on bovine milk fat composition. Anim. Genet. 40, 909–916.

Soyeurt, H., Gengler, N., 2008. Genetic variability of fatty acids in bovine milk. Biotechnol. Agron. Soc. Environ. 12, 203–210.

Viitala, S., Szyda, J., Blott, S., Schulman, N., Lidauer, M., Mäki-Tanila, A., Georges, M., Vilkki, J., 2006. The role of the bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in Finnish Ayrshire dairy cattle. Genetics 173, 2151–2164.

Xu, H.E., Lambert, M.H., Montana, V.G., Parks, D.J., Blanchard, S.G., Brown, P.J., 1999. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. Mol. Cell 3, 397–403.

Zidi, A., Fernández- Cabanás, V.M., Urrutia, B., Carrizosa, J., Polvillo, O., González-Redondo, P., Jordana, J., Gallardo, D., Amills, M., Serradilla, J.M., 2010. Association between the polymorphism of the goat stearoyl-CoA desaturase 1 (SCD1) gene and milk fatty acid composition in Murciano-Granadina goats. J. Dairy Sci. 93 (9), 4332–4339.