Milk fatty acid profile is modulated by *DGAT1* and *SCD1* genotypes in dairy cattle on pasture and strategic supplementation

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ABSTRACT. Milk fat composition is important to consumer health. During the last decade, some fatty acids (FA) have received attention because of their functional and beneficial effects on human health. The milk FA profile is affected by both diet and genetics. Differences in milk fat composition are based on biochemical pathways, and candidate genes have been proposed to explain FA profile variation. Here, the association between *DGAT1* K232A, *SCD1* A293V, and *LEPR* T945M markers with milk fat composition in southern Chile was evaluated. We selected five herds of Holstein-Friesian, Jersey, Frisón Negro, Montbeliarde, and Overo Colorado cows (pasture-grazed) that received strategic supplementation with concentrates and conserved forages. We genotyped the SNPs and calculated allele frequencies and

Hardy-Weinberg equilibrium. Milk fat composition was determined for individual milk samples over a year, and associations between genotypes and milk composition were studied. The most frequent variants for *DGAT1*, *SCD1*, and *LEPR* polymorphisms were GC/GC, C, and C, respectively. The *DGAT1* GC/GC allele was associated with lower milk fat and protein content, lower saturated fatty acid levels, and higher polyunsaturated FA (PUFA), *n*-3 and *n*-6 FA, and a linolenic acid to cholesterolemic FA ratios, which implied a healthier FA profile. The *SCD1* CC genotype was associated with a low cholesterolemic FA content, a high ratio of linolenic acid to cholesterolemic FA, and lower conjugated-linolenic acid and PUFA content. These results suggest the possible modulation of milk fat profiles, using specific genotypes, to improve the nutritional quality of dairy products.

Key words: Dairy cattle; Milk fat composition; Genetic markers; Conjugated-linolenic acid; *DGAT1*; *SCD1*

INTRODUCTION

Regarding human nutrition, bovine milk is one of the most important foods because it is a rich source of protein, fat, and calcium. Recently, milk fatty acids (FA) such as butyrate, conjugated-linolenic acid (*cis*-9, *trans*-11 CLA or rumenic acid), and *n*-3 and *n*-6 FA have been associated with improved milk quality, thus coining the term "functional milk" (Haug et al., 2007).

Milk fat is a complex source of lipids, including tri- and diacylglycerols, phospholipids, and cholesterol. Although more than 400 FA have been identified in milk, only twelve are represented at levels greater than 1% of the total milk lipid content (Jensen, 2000). These compounds range from C4 to C18 and include saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) FA.

In dairy cows, FA that are incorporated into milk triacylglycerol (TAG) are derived from two sources, *de novo* FA biosynthesis in the mammary gland and the uptake of preformed FA from peripheral circulation (Palmquist et al., 2005). In the mammary gland, acetate and 3-hydroxybutyrate, absorbed from the rumen, are the substrates for the *de novo* synthesis of short- and medium-chain FA (C4 to C16) (Shingfield et al., 2010), and these FA represent 40 to 50% of the milk FA. The remaining milk FA are medium- and long-chain FA (C16 to 2) that were taken up from triglycerides in plasma chylomicrons and very low-density lipoproteins or those that were bound to albumin (Bernard et al., 2008). These FA are of dietary origin, or they result from either ruminal fermentation (either modified or unchanged) or adipose tissue mobilization.

The degree of milk fat unsaturation is modulated by nutrition (Chilliard et al., 2000; Bauman and Griinari, 2003; Bernard et al., 2008). Other factors that regulate the milk fat profile are age, stage of lactation, and genetics (Jensen, 2002). Several authors describe differences among breeds in terms of milk composition, FA profile, and the degree of unsaturation (Lawless et al., 1999; Kelsey et al., 2003; Soyeurt et al., 2006; Arnould and Soyeurt, 2009). In addition, several molecular markers (e.g., SNPs) have been associated with milk fat content and FA composition. However, a small number of reports, including those focused on individual milk samples, have been evaluated because of the high cost of FA profile analyses. The most important reported marker is *DGAT1* K232A, which explains 50% of the

variation in milk fat content (Grisart et al., 2002; Schennink et al., 2007). *DGAT1* encodes diacylglycerol acyl-CoA acyltransferase I that catalyzes the esterification of a fatty acyl-CoA to the sn-3 position of a diacylglycerol, which is a key step in triglyceride biosynthesis (Cases et al., 1998). Other interesting genetic markers are *SCD1* A293V and *LEPR* T945M. *SCD1* encodes delta-9 fatty acid desaturase, which is an important enzyme involved in MUFA and CLA local biosynthesis in the mammary gland (Bauman and Griinari, 2003; Flowers and Ntambi, 2008). *LEPR* encodes the leptin receptor, a widespread expressed protein, which regulates homeostasis and the partitioning of energy and fat deposits in tissues, regulating milk fat and protein synthesis (Chilliard et al., 2005; Komisarek and Dorynek, 2006).

Approximately 75% of the milk in Chile is produced in the south area (the Los Lagos and Los Ríos regions) under grazing conditions (ODEPA, 2014). Recent evidence indicated that milk fat from grazing cows in this area had higher FA concentrations, which were beneficial to human health, than the milk from cows fed total mixed rations (Morales et al., 2015). In these regions, several dairy breeds are utilized, including Holstein-Friesian, Jersey, and other genotypes. However, there is no information regarding the relationship between milk composition and genetic marker variants in southern Chile. The objective of this study was to evaluate the association between *DGAT1* K232A, *SCD1* A293V, and *LEPR* T945M polymorphisms and milk production, fat content, and FA profiles in different dairy cattle present in southern Chile.

MATERIAL AND METHODS

Farms and animal selection

All animal procedures were performed according to requirements established by the Bioethics Committees of the Instituto de Investigaciones Agropecuarias (INIA) and the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT). Five dairy farms that produced milk using Holstein-Friesian (HF), Jersey (J), Montbeliarde (MB), Frisón Negro (FN), and Overo Colorado (OC) breeds were selected from a total of 15 surveyed farms. Approximately 50 to 60 cows with two to four lactations were selected from each farm, and all had less than 10% Holstein genetic backgrounds (with the exception of HF) according to genealogic records. All farms were located in the central valley area of the Los Ríos region, and official production records were available. All farms were of similar size, and they used comparable management practices (year-round grazing and milking frequency) and feeds.

Sampling and records

Each farm was visited every 2 months from July 2012 to July 2013. During the first visit, single blood samples (4 mL) were obtained from cows by caudal puncture using a Vacutest plast system (KIMA, Italy) with the anticoagulant ethylenediaminetetraacetic acid, and refrigerated samples were transported to the laboratory. Individual milk samples (200 mL), representing the morning and evening milk in equal parts, were collected during each visit. Fifty milliliter sub-samples were stored at 4°C with a preservative (2-bromo-2-nitro-1,3-propanediol; Sigma-Aldrich, USA) for milk component analysis and somatic cell count. The remaining 150 mL was stored at -20°C for FA analysis. Milk production data and monthly dietary composition were obtained via interview questionnaires and farm records. The diet

was similar for all herds during the entire study, and it mainly consisted of fresh pastures (supplemented with conserved forage such as silage, hay, and maize during autumn and winter), minerals, and different amounts of concentrates that were offered in the parlor, which varied throughout the year (Table 1).

Table 1. Quantity of concentrate (kg/day) consumed by each breed during the study.

Breed	Jul-12	Sep-12	Nov-12	Jan-13	Mar-13	May-13	Jul-13
Holstein-Friesian	7.9	8.2	8.0	8.0	8.0	8.0	8.0
Jersey	5.9	4.5	4.5	4.5	4.5	4.5	4.5
Frisón Negro	5.0	4.0	4.0	4.0	3.0	3.0	2.5
Montbeliarde	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Overo Colorado	3.0	3.0	3.0	3.0	3.0	3.0	3.0

Genotyping

Genomic DNA was purified using the FavorPrep™ Blood Genomic DNA Extraction Mini Kit (Favorgene, USA) following the manufacturer protocol. Briefly, 200 µL blood was lysed in a digestion buffer containing RNase (20 mg/mL) and proteinase K, and it was then purified in an affinity column. DNA was quantified in triplicate by fluorescence in a Rotor-Gene™ 6000 platform (Corbett Life Science, Australia) using the Quant-iT™ PicoGreen® dsDNA kit (Invitrogen, USA), and its integrity was assessed using agarose gel electrophoresis. Isolated DNA was stored at -20°C for subsequent analyses. Samples were genotyped by PCR-RFLP. Briefly, genomic DNA was amplified via PCR using specific primers based on published sequence data (GenBank accession Nos. AY065621, AY241932, and AJ580801; Table 2). The reactions were carried out in 15-µL volumes containing 5 ng DNA, 1X PCR buffer, 0.3 to 0.5 µM of each primer (Table 2), 0.25 mM of each dNTP, 2 mM Mg^{2+}, and 1.5 U *Taq* polymerase (Fermentas, USA). The thermal cycling conditions were as follows: an initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 45 s, annealing for 45 s, 72°C for 60 s, and a final extension at 72°C for 5 min. The amplicon specificity was corroborated using commercial sequencing (Macrogen, Korea). Digestion of 10-µL PCR product was performed overnight at 37°C in a 10-µL volume with specific restriction enzymes (2.5 U; Table 2), following the manufacturer protocol (Fermentas), and the results were visualized on 3% agarose gels with GelRed (Biotium, UK) staining using a UV-light and gel imaging system (UVP PhotoDoc-It, USA).

Genetic analyses

After genotyping, genetic and allelic frequencies of SNPs were calculated by simple count, and Hardy-Weinberg equilibrium (HWE) was determined using the chi-square test at $P < 0.05$ (Falconer and Mackay, 1996). Due to the lack of orthogonality of allele frequencies for *LEPR* T945M among herds, this marker was not considered for association analyses.

Table 2. Marker, primers, and conditions for PCR-RFLP genotyping.

Marker	Primer	Tm	Enzyme	Digested products (bp)
DGAT1 K232A	F: 5'-TGCCGCTTGCTCGTAGCTTTGGCC-3	60°C	*Cfr*I	AA/AA: 414 GC/AA: 414, 210, 204 GC/GC: 210, 204
	R: 5'-ACCTGGAGCTGGGTGAGGAACAGC-3			
SCD1 A293V	F: 5'-GGATACCGCCCTTATGACAA-3	56°C	*Fau*I	TT: 417, 308 CT: 417, 308, 240, 177 CC: 308, 240, 177
	R: 5'-AATACCCTAAGCAGCAGACC-3			
LEPR T945M	F: 5'-GCAACTACAGATGCTCTACTTTTGT-3	56°C	*Taq*I	TT: 400 CT: 400, 375, 25 CC: 375, 25
	R: 5'-CAGGGAAATTTCCCTCAAGTTTCAA-3			

F: forward primer; R: reverse primer; Tm: annealing temperature.

Fatty acid analyses

Milk fat, protein, and lactose were analyzed using near infrared spectroscopy (MILKOSCAN™, Foss Electrics, Denmark), and somatic cells were counted with a FOSSOMATIC™ analyzer at the laboratory of milk quality of INIA Carillanca. Milk fat extraction was conducted according to Morales et al. (2015). Briefly, milk FA were methylated based on the methods of Ichihara et al. (1996), and were subsequently analyzed using gas chromatography (GC 2010 Plus equipped with an FID detector; Shimadzu, Japan). Separation was performed using a capillary column (SPtm-2560, 100 m x 0.25 mm x 0.2 µm; Sigma-Aldrich) with an initial oven temperature of 140°C, which was increased at 4°C/min until a temperature of 240°C was reached. Injector and detector temperatures were set at 260°C. Helium was used as carrier gas at a flow rate of 0.5 mL/min, and the injector split was set at 100:1. A mixture of FA methyl esters (Supelco 37 Component FAME Mix; Supelco Analytical, USA), CLA ethyl ester (9c, 11 tr-Octadecadienoic; Larodan Fine Chemicals, Sweden), *trans*-11-vaccenic methyl ester (Perkin Elmer, USA), and PUFA-2 (Supelco Analytical) were used as external standards. C19 (NU-Check Prep, Inc., Elysian, USA) was added before extraction as an internal standard.

Statistical analysis

The associations between SNPs and milk production, fat and protein content, and milk fat composition were analyzed using a mixed model including the genotype for each of the genes studied, number of parity (NOP), days in milk (dim), sampling date, and the double interactions between genotype and each NOP, dim and sampling date, as fixed effects. The random effect of cow, with each cow nested within its corresponding genotype was included as a random effect. When the genotype effect was significant ($P < 0.05$), least-square means were separated using the Tukey HSD test. Statistical analyses were conducted with JMP® 11.0.0 (SAS Institute Inc.).

RESULTS

Allele frequencies and milk parameters

Allele frequencies for *DGAT1* K232A, *SCD1* A293V, and *LEPR* T945M polymorphisms in dairy cattle are shown in Table 3. The most frequent variant of the

DGAT1 K232A marker in all breeds (with the exception of J), was the GC allele (also known as variant A). In J, the AA allele (variant K) was predominant. This locus was at HWE (P > 0.08) in HF, FN, J, and MB herds but not in OC (Table 3). Genotype AA was associated with high milk content of total solids (9.6 versus 7.3% for AA/AA and GC/GC, respectively; Table 4), In accordance, milk from J cows that carry the AA/AA genotype more frequently had higher fat (5.11%) and protein (3.94%) content (Figure 1B and C). However, we did not find an association between the *DGAT1* K232A genotypes and milk yield. Conversely, milk from HF cows (frequently carry the GC/GC genotype) had lower total solid levels during the evaluated months (Figure 1B and C). In addition, these cows showed a higher milk production (Figure 1A).

For the *SCD1* A293V marker, all of the herds were at HWE (P > 0.05) and in all five breeds, the C allele was the most frequent allele (Table 3). We found that bovines with the CC genotype had a lower milk production level, but there was no association with fat or protein content (Table 4).

The most frequent variant for *LEPR* T945M in all breeds was the C allele, and HF, FN, and OC only had the CC or CT genotypes (Table 3). In addition, only the HF, FN, and OC herds were at HWE (P > 0.05; Table 3).

Table 3. Allele frequencies for *DGAT1* K232A, *SCD1* A293V, and *LEPR* T945 M polymorphisms in dairy cattle.

Breed	N	*DGAT1* K232A			*SCD1* A293V			*LEPR* T945M		
		GC	AA	χ^2	C	T	χ^2	C	T	χ^2
Holstein	58	0.66	0.34	0.84	0.65	0.35	1.67	1.00	0.00	0.00
Jersey	51	0.28	0.72	2.13	0.81	0.19	0.04	0.96	0.04	50.0*
Frisón Negro	52	0.97	0.03	0.05	0.89	0.11	0.73	1.00	0.00	0.00
Montbeliarde	50	0.96	0.04	0.09	0.56	0.44	0.93	0.96	0.04	49.0*
Overo Colorado	59	0.73	0.27	4.44*	0.92	0.08	0.51	1.00	0.00	0.00

GC, AA, C, and T: alleles; χ^2: chi-square test; *P < 0.05.

Milk fat composition

Table 5 summarizes the association between the *DGAT1* K232A and *SCD1* A293V markers and the milk FA profile. The GC/GC genotype of *DGAT1* K232A was associated with lower SFA content and higher PUFA and *n*-3 and *n*-6 FA content. In addition, this genotype was associated with a higher content of linolenic acid and lower levels of cholesterolemic FA (sum of C12, C14, and C16) resulting in a high ratio between them. In contrast, the genotype AA/AA was associated with higher SFA content and lower PUFA and *n*-3 and *n*-6 FA levels, and a lower ratio of linolenic acid and cholesterolemic FA (Table 5). We did not find associations between this marker and others FA as C4, C16:1, CLA c9 tr 11 or MUFA.

For the *SCD1* A293V marker we found that CC genotype (variant V) was associated with lower milk CLA and PUFA content. No differences were found for *n*-6:*n*-3 ratio but all genotypes exhibited values below 4.0 (Table 5; as well to *DGAT1* marker). In addition, this genotype was associated with lower levels of cholesterolemic FA resulting in a high ratio between linolenic acid and them.

Table 4. *DGAT1* K232A and *SCD1* A293V polymorphisms, milk production, fat, and protein content.

	DGAT1 K232A			*SCD1* A293V		
	GC/GC	GC/AA	AA/AA	CC	CT	TT
Milk (L/day)	20.8 ± 0.82[a]	22.3 ± 1.05[a]	20.3 ± 2.35[a]	19.3 ± 0.70[b]	23.4 ± 0.91[a]	22.4 ± 2.47[ab]
Fat (%)	3.78 ± 0.09[c]	4.37 ± 0.14[b]	5.68 ± 0.24[a]	4.25 ± 0.097[a]	3.96 ± 0.12[a]	4.11 ± 0.32[a]
Protein (%)	3.50 ± 0.03[b]	3.70 ± 0.05[a]	3.92 ± 0.09[a]	3.66 ± 0.033[a]	3.60 ± 0.041[a]	3.54 ± 0.01[a]

[ab]Different letters within a row indicate significant differences among genotypes ($P < 0.05$).

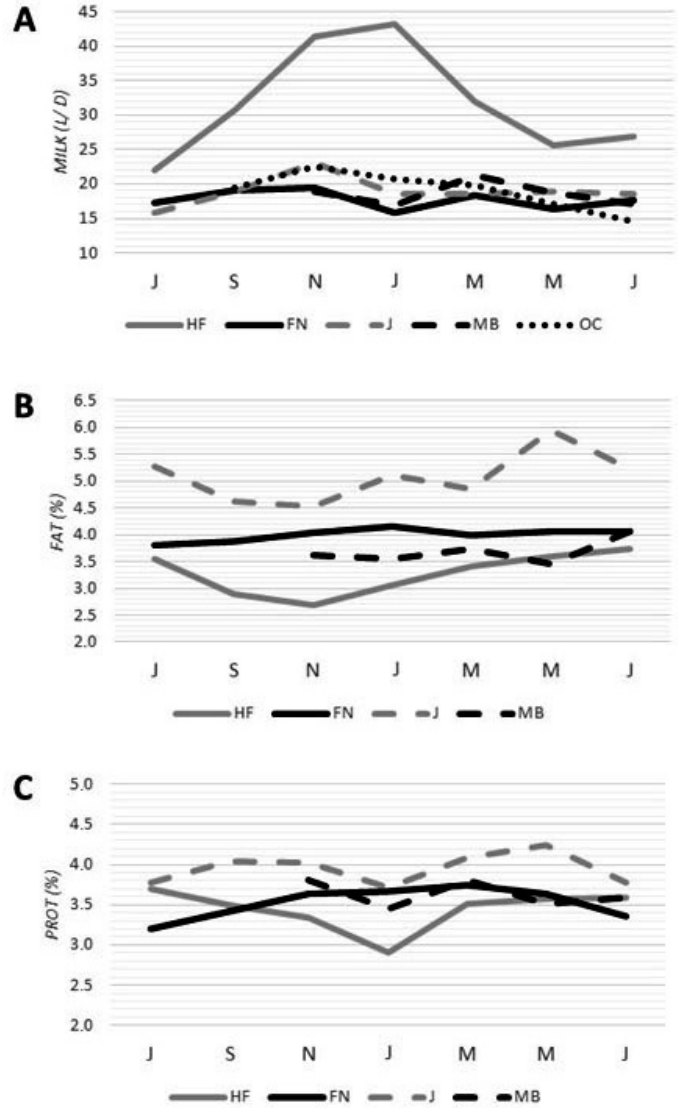

Figure 1. Time-course of milk production and milk total solids (fat and protein) by breed. HF = Holstein-Friesian; FN = Frisón Negro; J = Jersey; MB = Montbeliarde; OC = Overo Colorado. J = July; S = September; N = November; J = January; M = March; M = May; J = July; respectively, as shown in abscissae.

Table 5. Associations between *DGAT1* K232A and *SCD1* A293V genotypes and milk fat composition in dairy cattle (least square means ± SE).

		DGAT1			*SCD1*	
FA	GC/GC	GC/AA	AA/AA	CC	CT	TT
C4	1.84 ± 0.03^{a}	1.84 ± 0.04^{a}	1.90 ± 0.10^{a}	1.81 ± 0.03^{a}	1.86 ± 0.04^{a}	1.80 ± 0.12^{a}
C6	1.28 ± 0.02^{b}	1.38 ± 0.03^{a}	1.39 ± 0.06^{ab}	1.32 ± 0.02^{b}	1.31 ± 0.02^{b}	1.62 ± 0.07^{a}
C8	0.95 ± 0.02^{b}	1.03 ± 0.02^{a}	1.03 ± 0.04^{ab}	0.98 ± 0.01^{b}	0.98 ± 0.02^{b}	1.19 ± 0.05^{a}
C10	2.46 ± 0.04^{b}	2.69 ± 0.05^{a}	2.62 ± 0.12^{ab}	2.51 ± 0.04^{b}	2.60 ± 0.05^{b}	3.12 ± 1.14^{a}
C11	0.31 ± 0.02^{b}	0.35 ± 0.01^{a}	0.95 ± 0.02^{ab}	0.32 ± 0.01^{b}	0.32 ± 0.01^{b}	0.44 ± 0.03^{a}
C12	3.18 ± 0.05^{b}	3.43 ± 0.06^{a}	3.27 ± 0.15^{ab}	3.22 ± 0.04^{b}	3.36 ± 0.06^{ab}	3.69 ± 0.17^{a}
C13	0.18 ± 0.02^{a}	0.21 ± 0.02^{a}	0.17 ± 0.05^{a}	0.19 ± 0.02^{a}	0.19 ± 0.02^{a}	0.21 ± 0.06^{a}
C14	11.2 ± 0.09^{b}	11.7 ± 0.12^{a}	11.1 ± 0.27^{ab}	11.1 ± 0.08^{c}	11.6 ± 0.11^{b}	12.4 ± 0.30^{a}
C15	1.22 ± 0.02^{a}	1.18 ± 0.02^{a}	1.06 ± 0.04^{b}	1.19 ± 0.01^{a}	1.18 ± 0.02^{a}	1.17 ± 0.05^{a}
C16	30.3 ± 0.27^{a}	31.0 ± 0.34^{a}	31.7 ± 0.78^{a}	30.6 ± 0.23^{b}	30.6 ± 0.31^{b}	32.8 ± 0.85^{a}
C17	0.60 ± 0.01^{a}	0.62 ± 0.02^{a}	0.53 ± 0.04^{a}	0.60 ± 0.01^{a}	0.60 ± 0.02^{a}	0.55 ± 0.04^{a}
C18	12.7 ± 0.23^{ab}	12.1 ± 0.29^{b}	14.3 ± 0.66^{a}	13.3 ± 0.19^{a}	12.1 ± 0.25^{b}	10.2 ± 0.70^{c}
C20	0.17 ± 0.00^{a}	0.15 ± 0.00^{b}	0.18 ± 0.01^{ab}	0.17 ± 0.00^{a}	0.16 ± 0.00^{b}	0.14 ± 0.01^{b}
C22	0.08 ± 0.04^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.09 ± 0.00^{a}	0.09 ± 0.00^{a}	0.08 ± 0.02^{a}
C24	0.05 ± 0.00^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.04 ± 0.02^{a}
C14:1	1.03 ± 0.02^{ab}	1.07 ± 0.03^{a}	0.88 ± 0.06^{b}	1.02 ± 0.02^{a}	0.99 ± 0.03^{a}	0.97 ± 0.07^{a}
C16:1	1.20 ± 0.03^{a}	1.28 ± 0.04^{a}	1.30 ± 0.09^{a}	1.14 ± 0.03^{c}	1.28 ± 0.03^{b}	1.57 ± 0.09^{a}
C17:1	0.26 ± 0.01^{a}	0.25 ± 0.01^{a}	0.25 ± 0.022^{a}	0.24 ± 0.01^{a}	0.26 ± 0.01^{a}	0.23 ± 0.02^{a}
C18:1N7	0.12 ± 0.01^{a}	0.14 ± 0.01^{a}	0.13 ± 0.00^{a}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.16 ± 0.02^{a}
C18:1N9C	25.1 ± 0.28^{a}	24.0 ± 0.36^{a}	23.4 ± 0.82^{a}	24.6 ± 0.25^{a}	24.2 ± 0.34^{a}	21.4 ± 0.94^{b}
C18:1N11TC	3.60 ± 0.19^{a}	2.86 ± 0.23^{b}	2.96 ± 0.55^{ab}	3.39 ± 0.17^{a}	3.49 ± 0.22^{a}	2.98 ± 0.53^{a}
C22:1N9	0.07 ± 0.02^{a}	0.09 ± 0.02^{a}	0.06 ± 0.04^{a}	0.09 ± 0.02^{a}	0.06 ± 0.03^{a}	0.07 ± 0.09^{a}
C18:2N6C	1.31 ± 0.04^{a}	1.37 ± 0.06^{a}	1.17 ± 0.01^{a}	1.17 ± 0.04^{b}	1.47 ± 0.05^{a}	1.52 ± 0.13^{a}
C18:2N6T	0.30 ± 0.01^{a}	0.33 ± 0.02^{a}	0.28 ± 0.04^{a}	0.28 ± 0.01^{a}	0.32 ± 0.02^{a}	0.34 ± 0.04^{a}
CLA 9c 11tr	1.34 ± 0.04^{a}	1.30 ± 0.05^{a}	1.11 ± 0.11^{a}	1.23 ± 0.03^{b}	1.50 ± 0.04^{a}	1.52 ± 0.12^{a}
C18:3N3	0.86 ± 0.02^{a}	0.78 ± 0.02^{b}	0.70 ± 0.05^{b}	0.84 ± 0.01^{a}	0.83 ± 0.02^{ab}	0.72 ± 0.05^{b}
C18:3N6	0.20 ± 0.01^{a}	0.14 ± 0.02^{b}	0.13 ± 0.03^{ab}	0.84 ± 0.01^{a}	0.83 ± 0.02^{a}	0.71 ± 0.05^{a}
Others	14.7 ± 9.36^{a}	0.71 ± 12.40^{a}	0.54 ± 27.30^{a}	9.62 ± 7.80^{a}	0.59 ± 10.70^{a}	0.58 ± 31.60^{a}
SFA	66.4 ± 0.33^{b}	67.5 ± 0.42^{ab}	69.3 ± 0.97^{a}	67.3 ± 0.30^{a}	66.8 ± 0.40^{a}	69.2 ± 1.10^{a}
MUFA	29.0 ± 0.29^{a}	27.9 ± 0.37^{a}	27.1 ± 0.84^{a}	28.3 ± 0.26^{a}	28.3 ± 0.35^{a}	26.5 ± 0.97^{a}
PUFA	3.99 ± 0.08^{a}	3.89 ± 0.10^{ab}	3.37 ± 0.22^{b}	3.68 ± 0.07^{b}	4.28 ± 0.09^{a}	4.16 ± 0.24^{ab}
n-6	0.20 ± 0.01^{a}	0.14 ± 0.02^{a}	0.13 ± 0.03^{a}	0.19 ± 0.01^{a}	0.16 ± 0.01^{a}	0.17 ± 0.04^{a}
n-6:*n*-3	2.39 ± 0.12^{a}	2.75 ± 0.15^{a}	2.47 ± 0.34^{a}	2.13 ± 0.10^{a}	2.67 ± 0.13^{a}	3.47 ± 0.37^{a}
C14:1/C14:0	8.27 ± 0.22^{a}	8.50 ± 0.28^{a}	7.12 ± 0.63^{a}	8.42 ± 0.19^{a}	7.66 ± 0.25^{b}	7.20 ± 0.72^{ab}
C16:1/C16:0	3.99 ± 0.09^{a}	4.12 ± 0.12^{a}	4.21 ± 0.27^{a}	3.72 ± 0.08^{a}	4.22 ± 0.10^{aa}	4.74 ± 0.29
MGS	36.6 ± 0.27^{b}	38.1 ± 0.35^{a}	37.7 ± 0.80^{ab}	36.8 ± 0.23^{b}	37.6 ± 0.31^{b}	40.8 ± 0.88^{a}
MCLMGSFA	13.5 ± 0.02^{a}	13.5 ± 0.02^{a}	13.5 ± 0.05^{a}	36.8 ± 0.23^{a}	37.6 ± 0.31^{a}	40.8 ± 0.88^{a}
RA:CHFA	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{b}	0.04 ± 0.00^{a}	0.03 ± 0.00^{ab}
LNA:CHFA	0.02 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{b}

FA = fatty acid; CLA 9c 11tr = rumenic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; *n*-6:*n*-3 = proportion *n*-6:*n*-3; Index C14 = C14:1/C14; Index C16 = C16:1/C16; MGS = fatty acid synthetized in mammary gland; MCLMGSFA = mean chain length of saturated fatty acid synthetized in mammary gland; RA:CHFA = proportion rumenic acid/cholesterolemic acids (C12 + C14 + C16); LNA:CHFA = proportion linolenic acid/cholesterolemic acids (C12 + C14 + C16); ^{abc}Different letters within a row indicate significant differences among genotypes ($P < 0.05$).

DISCUSSION

The frequencies observed for the *DGAT1* K232A marker in HF, J, and MB were similar to previous reports (Spelman et al., 2002; Schennink et al., 2008; Berry et al., 2010). The association of the genotype AA with high milk content of total solids in our study has

been widely reported (Grisart et al., 2002, 2004; Gautier et al., 2007; Schennink et al., 2008). According to Winter et al. (2002) and Schennink et al. (2007), the presence of Lys in position 232K of *DGAT1* (variant K) is strongly associated with higher milk fat content and lower milk production, and this is due to an alteration in diacylglycerol acyl-CoA acyltransferase I kinetics and thus the biosynthesis of TAGs. Thus, J cows have more total solids in milk.

In contrast, HF cows, where predominates the variant A, produce less total solids (Prendiville et al., 2009). These differences in milk traits are a consequence of several years of genetic selection, which favor milk production in HF cows and protein content in J cows (Dekkers and Gibson, 1998). In relation to milk FA profile, we found an association between the GC/GC genotype of *DGAT1* K232A and lower SFA content and higher PUFA and *n*-3 and *n*-6 FA, and lower levels of cholesterolemic FA. Schennink et al. (2008) reported a significant association between this genotype and lower cholesterolemic FA and desaturation indices as well as higher CLA and C18 desaturation indices [(C18:1/C18) x 100]. In another study of Dutch HF cows, the same *DGAT1* variant (A) was associated with lower SFA and short- and medium-chain FA (Duchemin et al., 2013). Despite the fact that significant associations between *DGAT1* alleles and butyrate, CLA *cis*-9, *trans*-11 or MUFA were not found in our study, the milk fat composition of evaluated cows, which were mainly pasture-grazed, could be considered healthy based on current WHO recommendations (Lock et al., 2008; FAO, 2010). In this study, the milk contained low levels of C12, C14, and C16 and a desirable *n*-6:*n*-3 ratio (<4.0; Gómez Candela et al., 2011) for all genotypes. This highlights the possibility of the natural development of healthy dairy products.

For *SCD1* A293V, the genic frequencies were similar to what has been reported by Mele et al. (2007) and Moioli et al. (2007) with the predominance of the C variant. We found that the CC genotype was associated with less milk production, which is in agreement with those reported in the Macciotta et al. (2008) and Kgwatalala et al. (2009) studies in Holstein cows. Also, we associated this genotype with lower milk CLA and PUFA content, but lower levels of cholesterolemic FA. Schennink et al. (2008) reported lower levels of cholesterolemic FA and CLA in Holstein cows, which is in agreement with our results. However, different associations with the milk FA profile have been reported for this marker in the literature. For example, Mele et al. (2007) found higher levels of MUFA, SFA, and C14:1 and C18:1 *cis*-9 for variant A, while Moioli et al. (2007) and Kgwatalala et al. (2009) did not find differences. Among other factors, these different associations can be caused by sample size, different diets (e.g., the level of inclusion of PUFA such as omega-6 and omega-3), or the genetic background (pure biotypes). In our case, we selected herds with similar management and feeding systems that were based on pastures with grain supplementation during critical seasons when grass availability is restricted (e.g., summer and winter). In addition, while we tried to select pure cows based on genealogic information, it is generally recognized that available populations still correspond to mixed populations from different sources or germlines of each breed (e.g., North American and/or European Holstein or Irish and New Zealander Frisón). This made it difficult to identify genetically pure herds, to increase the sample size, and to determine the effect of markers in each breed.

Regarding *LEPR* marker, we did not find the T allele in HF, FN and OC herds, and only observed a very lower representation in J and MB cows. The low representation of the T allele is in agreement with previous reports (Carvajal, 2011). Due to this distribution, this marker was not considered for association analyses but other reports suggest some effects over milk production and composition (Komisarek and Dorynek, 2006).

Finally, it is important to highlight that, to our knowledge, the genic frequencies determined in the creole breeds (FN and OC) for the *DGAT1*, *SCD1,* and *LEPR* markers are the first reported.

Milk production is an important economic activity in southern Chile, and milk with a healthier FA profile presents an opportunity to improve farmer incomes by marketing dairy products with added value. This study provides information on how different genotypes found in dairy breeds available to farmers are associated with milk fat composition. Two creole breeds (FN and OC) were studied for the first time in this respect, and were compared to globally widespread breeds such as HF, J, and MB. Our results show some interesting associations between milk fat profiles and *DGAT1* and *SCD1* polymorphisms.

Conflicts of interest

The authors declare no conflict of interest.

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