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Gelsolin expression in sheep milk somatic cells during lactation

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The identification of genes involved in phenotypes related to milk quality is important for both economic and health aspects in livestock production. The aim of this study was to assess the level of gelsolin gene expression in two breeds of dairy sheep – Sarda and Gentile – with pronounced differences in quantitative and qualitative milk traits. Gelsolin, a type of actin-modulating proteins is involved in the processes of actin remodeling during cell growth and apoptosis; therefore a role of this protein in mammary changes during lactation was here hypothesized. Individual milk samples were collected three times during lactation from 26 ewes of the two breeds. The differential gene expression of gelsolin in the two breeds and the three lactation times was estimated by quantitative PCR on RNA extracted from milk somatic cells. Correlations of gelsolin gene expression with milk yield and quality and days of lactation were also estimated. The results showed that gelsolin gene expression was significantly higher in the Sarda compared to the Gentile at each lactation stage, in agreement with the longer lactation duration and the higher daily milk yield of the first breed. Significant correlations of gelsolin gene expression were found with milk fat content in Sarda breed (−0.46, $P < 0.05$). Gelsolin expression analysis confirmed the link between gelsolin gene function and milk fat content of sheep.

Keywords: dairy sheep, gene expression, gelsolin, milk fat, milk somatic cells

Implications

As with any other traits, milk quality is affected by environmental and animal genetics factors. Our study on gelsolin gene expression highlighted its link with milk fat content in sheep. Having identified a modulatory gene of milk fat content may lead to economic improvement both for breeders and dairy industry. Breeders can choose to select milk with lesser or greater fat content depending on the cost/benefit analysis. Process industries can indeed be inclined to prefer a higher fat content, not only for its greater yield, but especially for the finer cheese's organoleptic characteristics.

Introduction

Gelsolin is representative of a class of actin-modulating proteins found from lower eukaryotes through mammals. It is an ubiquitous and a multifunctional biomolecule, able to regulate cell structure and metabolism (Li *et al.*, 2012) and existing both as intrinsic cytoplasmic protein and as secreted plasma protein. Gelsolin cytoplasmic and secreted forms are the most potent actin filament-severing proteins observed until now (Spinardi and Witke, 2007). The actin cytoskeleton plays a pivotal role in several essential cellular processes involving force generation and movement easing (Li *et al.*, 2012). Actin role in cell shape and organization is relevant, consequently its function is crucial in a multiplicity of biochemical processes that mediate motility and/or structural reorganization within cell, for example, cell migration, development, morphogenesis, metastasis, cell–cell and cell–matrix adhesion and apoptosis (Nag *et al.*, 2013).

The assembly/disassembly and resulting organization of actin filaments in cells is controlled by interaction among a large number of distinct actin-binding proteins (ABPs), classified according to their functions of cross-linking, severing, capping and nucleating actin filaments. Gelsolin is an abundant ABP, and more specifically a Ca^{2+} and polyphosphoinositide 4,5-bisphosphate (PIP_2)-regulated actin filament severing and capping protein, involved in processes of actin remodeling (Sun *et al.*, 1999).

In mammalian, isoforms of gelsolin are encoded by a single gene (*GSN*) (chromosome 9 for *Homo sapiens*, chromosome 2 for *Mus musculus* and for *Ovis aries* and chromosome 8 for *Bos taurus*) are under the control of several promoters (Li *et al.*, 2012) and differ as a result of alternative initiation sites/splicing (Pottiez *et al.*, 2010). Nag *et al.* (2013) assumed that alternative splicing frequently observed in the 5′-untranslated regions (5′-UTR), leads to potential differences in transcription

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control, associated mRNA-binding proteins and mRNA localization. In *Bos taurus* there are three characterized gelsolin isoforms: a secretory plasma protein composed of 781 aa (isoform A encoded by transcript variant 1; accession number: NM_001113284) and two cytoplasmic proteins, isoform B composed of 731 aa (encoded by transcript variant 2; accession number: NM_001034627) and a lesser known isoform C composed of 742 aa (encoded by transcript variant X3; accession number: XM_015472727). Gelsolin exists in the blood plasma as an 83-kDa protein, while intracellularly it is present as an 81-kDa protein composed of six homologous subdomains referred to S1 to S6; all of these domains are important for the folding and enzymatic activity of the protein (Dos Remedios *et al*., 2003; Nag *et al*., 2013).

In our previous study (Signorelli *et al*., 2010) we produced evidence of the differential isoform B protein expression in the mammary glands of sheep that differed highly in terms of milk production traits. Addis *et al*. (2011) showed a significant overexpression of isoform B protein in milk fat globules of sheep naturally infected by a bacterial pathogen. In Napolitano *et al*. (2014) we identified the gelsolin gene as a candidate for milk quality and showed significant associations between some single nucleotide polymorphisms (SNPs) in intron 12 and milk fat content of Altamurana and Gentile sheep breeds. The aim of this study was to assess the level of *GSN* expression in two sheep breeds with pronounced differences in quantitative and qualitative milk traits and to verify if *GSN* cytoplasmic isoforms expression could influence milk fat as was already observed in previous genomic and proteomic studies.

Material and methods

Animals and Phenotyping

The trial was conducted in an experimental farm of the Council for Agricultural Research and Economics (CREA) located in South-Eastern Italy, following the recommendations of European Union directive 86/609/EEC. A total of 26 ewes of two sheep breeds (12 Gentile and 14 Sarda) were maintained on the same experimental farm and were traditionally managed. The ewes were housed on straw litter in boxes with outdoor pens. Feeding consisted of grazing whole day on natural pasture, with addition of 0.5 kg of a pellet concentrate (Mangimi Liverini S.p.A., Telese Terme (BN), Italy) and 1.8 kg of vetch/oat hay daily, provided as two meals per day (at 0730 and 1530 h). Natural pasture was composed with spontaneous grasses (mainly ryegrass perennial, orchard and fescue species), legumes (mainly trifolium) and others species (mainly chamomile, rocket salad and thistle). Pasture contributed to 20% of the daily diet. Water was available *ad libitum*. The chemical composition of diet and foods showed in Table 1 was analyzed by standard procedures (Association of Official Analytical Chemists, 1990), while NDF and ADF were determined according to Van Soest *et al.* (1991). Ewes were milked twice a day (at 0700 and 1500 h) from weaning (mid-April) until drying off (the end of July), using pipeline milking machines (Alfa Laval Agri, Tumba, Sweden).

Three times during lactation (60, 90 and 120 days) individual milk samples were collected in the morning milking and immediately transported to laboratory at 4°C for somatic cell recovery and RNA extraction. At the third lactation stage some sample were not collected because ewes had dried off. Individual milk production was measured through graduated measuring cylinders attached to individual milking units. Fat, total protein and lactose content was determined using a mid-IR spectrophotometer (MilkoScan FT120; Foss Electric, Hillerød, Denmark). Somatic cell count (SCC) was evaluated by a disk cytometry cell counter (Fossomatic 90; Foss Electric), following the International Committee for Animal Recording regulations.

Selection of reference genes for quantitative PCR and primer design

It is well known that the suitability of reference genes (RGs) for any type of quantitative PCR (qPCR) experiment is not given *a priori* but has to be evaluated each time (Hruz *et al*., 2011). Candidate RGs already used in a study on buffalo (Crisà *et al*., 2013) were tested: ATP synthase β polypeptide, nuclear gene encoding mitochondrial protein (*ATP5B*), Eukaryotic translation initiation factor eIF-2B subunit β (*EIF2B2*), succinate dehydrogenase complex, subunit A, flavoprotein (*SDHA*), DNA-directed RNA polymerase Fragment RNA polymerase II (*POLR2A*) and TATA-Box-binding protein (*TBP*). Moreover, we designed and tested new primers for three genes: ubiquitously expressed transcript (*UXT*), ribosomal protein S9 (*RPS9*), mitochondrial ribosomal protein S15 (*MRPS15*) by using Primer Express software

Table 1 *Chemical composition and nutritional value of the foods and diet of sheep*

	Diet ingredients (% DM)		
	Vetch and oat hay	Concentrate[1]	Pasture
Parameters			
DM	85.67	88.64	44.88
CP	12.07	19.09	10.20
Lipids	0.81	3.77	0.92
Crude fiber	35.22	7.87	31.56
NDF	60.50	32.61	66.90
ADF	39.66	9.52	29.86
Metabolizable energy	9.87	11.25	7.23
Diet composition			
DM	86.98		
CP	16.42		
Lipids	1.97		
Crude fiber	25.50		
NDF	55.19		
ADF	29.46		
Metabolizable energy	9.64		

[1]Ingredients: vitamin A, 1150 IU; vitamin D, 390 IU; vitamin E, 50 mg; vitamin PP, 8500 mg; vitamin B_1, 112 mg; vitamin B_2, 112 mg; vitamin B_6, 80 mg; vitamin B_{12}, 1 mg; D-Pantotenic acid, 2400 mg; Choline, 15 000 mg; Iron, 150 mg; Manganese, 800 mg; Zinc, 2200 mg; Cobalt, 8 mg; Iodine, 30 mg; Selenium, 5 mg; Molibdenum, 10 mg.

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(Life Technology). Primers were picked, when possible, intron-spanning and were synthesized by Eurofins MWG Operon (Ebersberg, Germany). Table 2 reports information relative to the primers used to amplify the target and RG and expected amplicon size. Preliminary amplifications were performed both on complementary DNA (cDNA) and DNA to validate the primers. Quality and length of PCR products were verified on an agarose gel run and after purification by Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA) were sequenced using the 3500 Genetic Analyzers (Applied Biosystems, Foster City, CA, USA). Amplification specificity was verified by using the BLAST software (Altschul *et al*., 1997).

RNA extraction and reverse transcription

Milk cells were pelleted using 50 ml of milk diluted 1 : 1 with Phosphate buffered saline (PBS) 1 × adding 50 µl of 0.5 M EDTA (pH = 8) to 50 ml and centrifuged at 2000 × **g** at 4°C for 10 min. Fat layer was removed from the top of the supernatant with a sterile pipette tip and the skimmed milk was discarded. The cell–pellet was washed with 8 ml of buffer (0.5 mM EDTA pH 8.0 in Dulbecco's PBS). After centrifugation, milk somatic cell pellet was resuspended with 1 ml TRI REAGENT (Sigma-Aldrich, Saint Louis, MO, USA) reagent and stored at −80°C (Crisà *et al*., 2016; Toral *et al*., 2016). Total RNA extraction was performed following manufacturer instructions and then was DNAse digested and cleaned with RNeasy Minelute kit (Qiagen, Venlo, The Netherlands). Ribonucleic acid quality and quantity was checked with spectrophotometric (NanoPhotometer™ Pearl; Implen, Los Angeles, CA, USA) and microfluidic (Bioanalyzer, Agilent, Santa Clara, CA, USA) inspections. Reverse transcription (RT) was performed starting from 1 µl of RNA (500 ng/µl) in a total volume of 20 µl containing 100 pmol oligo(dT) (18-mer), 0.5 mM Deoxynucleotide, 1 × RT buffer, RevertAid Premium Enzyme mix (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

Quantitative PCR

Preliminary qPCR experiments were performed to optimize the annealing temperature and primer concentration both for the target genes and for the eight RGs (Table 3).

Efficiency of PCR amplification for each gene was calculated with a five-point standard curve (1 : 5 dilution per point) made on a samples used as calibrator. For qPCR reactions, 10 ng cDNA from each sample was used in 20 µl reaction using the Max Sybr Green QPCR Master Mix (Thermo Scientific) onto the StepOne Plus instrument (Applied Biosystem). Each animals was tested in triplicate. Reverse transcript minus (RT −) and no template controls were included. The amplification protocol was composed of a denaturation step at 95°C for 10 min; 40 cycles of 30 s at 95°C, 30 s at specific annealing temperature (Table 3) and 1 min for elongation. A melting curve to assess amplicon specificity was included.

Data analysis

geNormplus analysis was assessed to screen the optimal set of RGs from eight tested candidate, using ten samples belonging to three lactation time (Vandesompele *et al*., 2002). geNorm calculates the gene expression stability measure (M) for a RG as the average 'pairwise variation' (V) for that same gene with all other tested RGs. Stepwise exclusion of the gene with the highest M value allows ranking of the tested genes according to their expression

Table 2 *Primers used to amplify the target and reference genes with respective amplicon size in sheep*

Gene symbol[1]	Sequence (5' to 3')	Accession number	Name/position	Amplicon (bp)
GSN	CCGCGTGCACGTTTCC	JF412292	Ex4fw/716-730	102
	TCCTTGGCTGTGTCCTCAGT		Ex5rw/798-817	
ATP5B	TTTGGACTCCACGTCTCGCATC	NM_175796.2	Ex8fw/1219-1240	108
	TCCTGGAGGGATTTGTAGTCCTG		Ex9rw/1304-1326	
EIF2B2	CCGTTCCCATTATGCTCAACTCCAG	NM_001015593.1	Ex3fw/395-419	81
	TCCGTTGTCCCTTCCAGTTCCAC		Ex3-4rw/453-475	
SDHA	ACGATTACTCCAAGCCCATCCAG	NM_174178.2	Ex14fw/1825-1847	80
	AACGTAGGAGAGCGTGTGCTTC		Ex14rw/1883-1904	
POLR2A	AATGGAAGCATGTCAATGAGGACTCTC	NM_001206313.1	Ex5fw/602-628	164
	CACAGGCAGCACAGTGACGATC		Ex5rw/744-765	
TBP	AACAGGTGCTAAAGTCAGAGCAG	NM_001075742.1	Ex8fw/1254-1276	102
	GGAGAACACAGCAGCCATTACG		Ex9rw/1334-1355	
UXT	TGTGGCCCTTGGATATGGTT	NM_001037471.2	Ex5fw/300-319	101
	GGTTGTCGCTGAGCTCTGTG		Ex5-6rw/381-400	
RPS9	CCTCGACCAAGAGCTGAAG	NM_001101152.1	Ex2fw/128-146	54
	CCTCCAGACCTCACGTTTGTTC		Ex3rw/170-191	
MRPS15	GCAGCTTATGAGCAAGGTCGT	NM_001192201.1	Ex5/324-344	151
	GCTCATCAGCAGATAGCGCTT		Ex7/454-474	

[1] *GSN* = gelsolin; *ATP5B* = ATP synthase β polypeptide; *EIF2B2* = eukaryotic translation initiation factor eIF-2B subunit β; *SDHA* = succinate dehydrogenase complex, subunit A, flavoprotein; *POLR2A* = DNA-directed RNA polymerase Fragment RNA polymerase II; *TBP* = TATA-Box-binding protein; *UXT* = ubiquitously expressed transcript; *RPS9* = ribosomal protein S9; *MRPS15* = mitochondrial ribosomal protein S15.

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Table 3 *Primer concentration and annealing temperature (T_a) used in the quantitative PCR in sheep with some parameters calculate from gene expression normalization analysis*

Gene symbol[1]	Primer nM	Slope	*Y*-intercept	R^2	Efficiency %	T_a
AAT	300/300	−3.27	27.50	0.99	102	58
GSN	300/300	−3.30	21.78	0.99	100	58
ATP5B	200/200	−3.10	19.44	0.99	104	61
EIF2B2	200/100	−3.26	30.38	0.96	102	61
POLR2A	100/100	−3.38	20.44	0.98	97.7	61
SDHA	200/100	−3.37	28.52	0.98	97.9	61
TBP	100/300	−3.39	29.18	0.96	97	58
UXT	300/300	−3.35	28.62	0.99	98.9	58
RPS9	200/200	−3.37	24.26	1	98.2	58
MRPS15	300/300	−3.44	29.59	0.99	95	58

[1]*AAT* = α 1 antitrypsin; *GSN* = gelsolin; *ATP5B* = ATP synthase β polypeptide; *EIF2B2* = eukaryotic translation initiation factor eIF-2B subunit β; *SDHA* = succinate dehydrogenase complex, subunit A, flavoprotein; *POLR2A* = DNA-directed RNA polymerase Fragment RNA polymerase II; *TBP* = TATA-Box-binding protein; *UXT* = ubiquitously expressed transcript; *RPS9* = ribosomal protein S9; *MRPS15* = mitochondrial ribosomal protein S15.

stability. The *GSN* expression analysis were performed by using the qBase^{PLUS} software based on the $2^{-\Delta\Delta Ct}$ method with implementations (Hellemans *et al.*, 2007; http://qbaseplus.com). Relative quantity values represent the *n*-fold normalized expression relative to inter run calibrators run across all plates. Calibrated and normalized log transformed relative quantity values from 26 ewes at each lactation time were used to estimate the different expression level of *GSN* in the two breeds. The repeated measure analysis in GLM procedure of SAS software (SAS Institute Inc., 2010) was used with the following equation:

$$Y_{ijkl} = \mu + A_i + B_j + (A \times B)_{ij} + b_{ik} + e_{ijkl}$$

where Y_{ijkl} was the level of expression of the *GSN*; A_i was the fixed effect of breed (i = Gentile, Sarda); B_j was the fixed effect at the time of the control (j = 60, 90, 120 d); b_{ik} was the random effect of subject within the breed i (k = 1, … , 14) and e_{ijkl} was the random error. The significance of the *GSN* expression level was determined using Student's *t*-test in the GLM procedure.

Correlation coefficients between lactation length, milk yield, fat content, protein content, lactose content, SCC and the *GSN* expression level only in Sarda milk samples at 120 days (since at that time of control Gentile milk was just enough to extract a satisfactory amount of RNA) were calculated using the CORR procedure of SAS to assess whether the changes in these phenotypic traits were proportional to the changes in *GSN* expression level. We chose to use Kendall's τ-b rank since the *P*-values are more accurate with smaller sample sizes, moreover, correlations with *P*-values <0.05 and $\tau\text{-}b > 0.46$ were considered of interest.

Results

In this work *GSN* expression level in two sheep breeds and in three lactation stages was investigated. The complexity of the *GSN* is well known, especially for alternative splicing frequently observed in the 5′-UTR, which determines the translation of three distinct isoforms. The simultaneous expression of both cytoplasmic isoforms was analyzed using primers drawn in conserved region of isoforms (between exons 4 and 5). We identified some candidate genes to use as reference and designed the relative primers basing on the *Bos taurus* sequence. At first we found that all genes were expressed in sheep milk somatic cell and the amplicons size were as expected both on cDNA (Table 2) and DNA (data not shown). Subsequently primer concentration and qPCR efficiency parameters were optimized (Table 3). Specific peaks were obtained from the dissociation analysis of all genes. To identify the most stable expressed RGs to be used in qPCR

Table 4 *Estimated means of the level of expression of the gelsolin in two breeds at different time of control in sheep milk*

Breeds		Gentile	Sarda				
	N[1]	26	40				
	μ ± SE	1.58 ± 0.070[b]	1.91 ± 0.046[a]				
Time of control		60days	90 days	120 days			
	N[2]	26	23	17			
	μ ± SE	1.53 ± 0.056[b]	1.85 ± 0.063[a]	1.86 ± 0.094[a]			
Breed		Gentile	Sarda	Gentile	Sarda	Gentile	Sarda
Time of control	N[3]	12	14	10	13	4	13
60 days	μ ± SE	1.26 ± 0.083[b]	1.80 ± 0.077[a]				
90 days	μ ± SE			1.63 ± 0.097[b]	2.08 ± 0.081[a]		
120 days	μ ± SE					1.86 ± 0.169	1.86 ± 0.081

[1] = Number of milk samples of each breed.
[2] = Number of milk samples of both breeds at each time of control.
[3] = Number of milk samples of each breed at different time of control.
μ = mean; SE = standard error of mean.
[a,b] = $P < 0.001$.

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experiment, a preliminary geNorm analysis (included in the qbase^{PLUS} software) showed that the optimal normalization factor could be calculated as the geometric mean of three reference targets (*ATP5B, SDHA* and *RPS9*).

Overall statistical analysis (Table 4) highlighted a significant ($P < 0.001$) difference between breeds, where Sarda showed a higher *GSN* expression compared to Gentile (1.91 *v.* 1.58). Considering the three lactation stages it significantly increased from 60 to 90 days (1.53 *v.* 1.85, $P < 0.001$), instead from 90 to 120 days it was invariable (1.85 *v.* 1.86). The higher expression in Sarda breed is statistically significant both at 60 days (1.26 *v.* 1.80, $P < 0.001$) and 90 days (1.63 *v.* 2.08, $P < 0.001$), after lambing, while at 120 days expression values were identical.

In Supplementary Tables were reported the descriptive statistics (Supplementary Table S1), the parametric and non-parametric rank correlations (Supplementary Tables S2 and S3, respectively) of the level of expression of the gelsolin and of the milk composition parameters in Sarda ewe. The analysis of Kendall's τ-b rank correlation coefficients between *GSN* expression and some milk traits (Table 5 and Figure 1) highlighted that to an increase of gene expression corresponds a significant reduction of milk fat content (−0.46, $P < 0.05$). Moreover, a significant correlation between lactose and somatic cell was observed (−0.52, $P < 0.01$).

Discussion

In a previous work the authors sequenced exons 12 and 13 and parts of introns 11, 12 and 13 in 263 sheep of three breeds (Altamurana, Gentile and Sarda) and showed that three SNPs were associated with fat content (Napolitano *et al*., 2014); moreover, these polymorphisms were located in the consensus sequences of binding sites for some transcription factors. Signorelli *et al*. (2010), in a study on comparative proteomic analysis of mammary gland on the same sheep breeds, observed the protein gelsolin isoform B downregulated in the Gentile breed compared to the Sarda one. Therefore the aim of this work was to verify a probable influence of *GSN* on milk fat content as already observed in previous genomic and proteomic studies. To verify that we chose two sheep breeds with opposite characteristics as for milk yield and fat content.

Table 5 *Kendall's τ-b rank correlation coefficients between productive traits and the level of expression of the gelsolin in Sarda ewe milk*

Traits	Lactation	Milk	Fat	Protein	Lactose	Log_{10} SCC
GSN	0.23	−0.01	−0.46*	−0.06	−0.36	0.33
Lactation (days)		0.15	0.21	0.09	0.13	−0.08
Milk (g)			−0.04	−0.21	0.33	−0.17
Fat (%)				0.30	0.26	−0.15
Protein (%)					0.06	0.22
Lactose (%)						−0.52**

GSN = gelsolin gene; SCC = somatic cell count.
*$P < 0.05$; **$P < 0.01$.

The obtained results highlighted a significant ($P < 0.001$) higher *GSN* expression in Sarda breed compared to Gentile and a negative correlation with milk fat percentage.

The connection between gelsolin and milk fat content could be explained through the synthesis and secretion of lipids by mammary epithelial cells (Figure 2; Bauman *et al*., 2006). As described by Bauman *et al*. (2006), microlipid droplets are released into the cytoplasm and coated with protein and polar lipids from the reticular membrane. Some of these droplets undergo a series of fusion reactions to form larger cytoplasmic lipid droplets during their transfer to the apex of the cell (pathway A in Figure 2). These droplets become relatively large before secretion, but many others are secreted virtually unaltered in size from the time of their initial formation (pathway B in Figure 2). The droplets therefore arrive at the apical surface with a cohort of surface proteins and polar lipids, some of which become components of the milk fat globule membrane (MFGM).

What guides this apical migration is not well known but it is probable that cytoskeleton components mainly actin are involved. In fact, actin has been localized to the cytoplasmic surface along basal regions of budding lipid droplets and its microfilaments are abundant in milk secreting cells and appear to be most concentrated in apical cell regions (Franke *et al*., 1980; Amato and Loizzi, 1981). Actin has been identified as a constituent of the MFGM and is an active participant in the milk fat secretory process (Heid and Keenan, 2005). We could suppose that gelsolin is involved in the synthesis of fat globules, as it is an actin filament severing and capping protein. Romao *et al*. (2014), in a study on bovine subcutaneous fat proteome, correlated *GSN* with fat metabolism and overall identified it among the few proteins to be associated with lipid transport and redistribution.

The authors highlight that the significant association found between the higher *GSN* expression level is at the same time parallel to the reduction of fat content. The authors hypothesize that this reduction could lead to a consequent lower milk globule fat transport and to resulting lower fat content in milk.

Somatic cell count and lactose negative correlation has long been known, in a review Pyörälä (2003) reported several previous studies focused on lactose as mastitis biomarker (PAF). Both because of the reduced synthesis capacity of damaged mammary tissue and the lower availability of glucose, due to competition for energy between secretor cells and polymorphonuclear leukocytes, lactose content decreases in ovine milk with intramammary infection (De Olives *et al*., 2013). These authors showed also fat and casein could vary in different way depending on the magnitude of milk yield reduction. Bianchi *et al*. (2004) comparing ovine milk samples from healthy and infected udders, highlighted a significant decrease both of lactose and fat.

This association between the two parameters was always confirmed over time (Berglund *et al*., 2007; Bobbo *et al*.,

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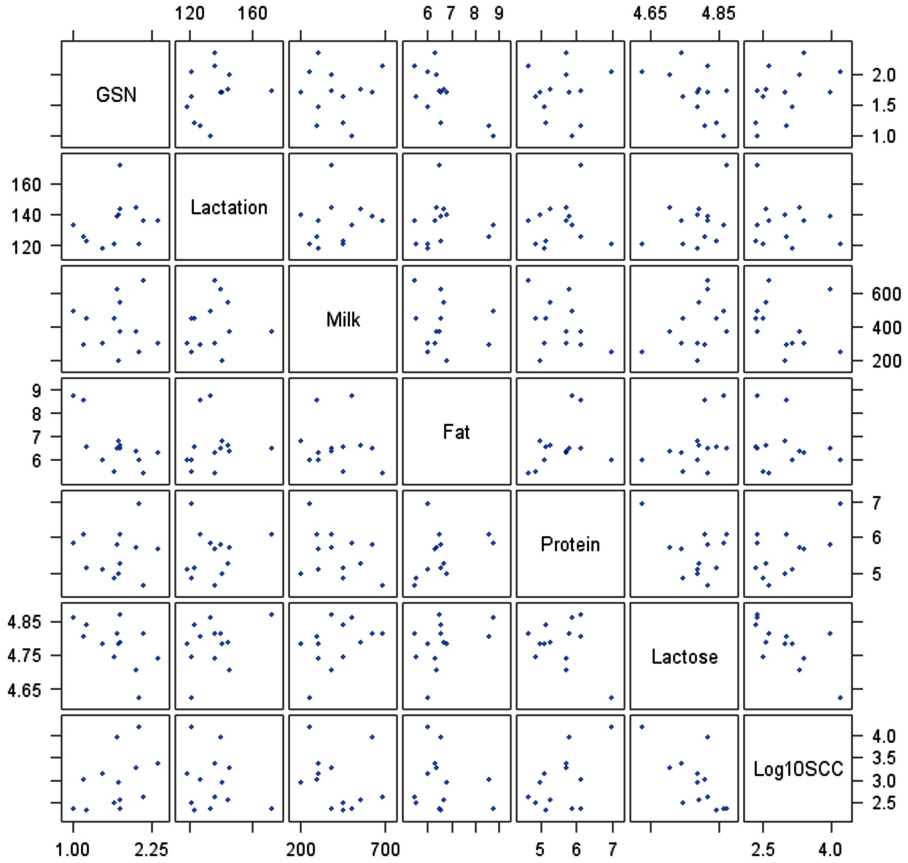


Figure 1 (colour online) Correlation plots between traits analyzed in Sarda ewe milk. *GSN* = gelsolin gene; SCC = somatic cell count.

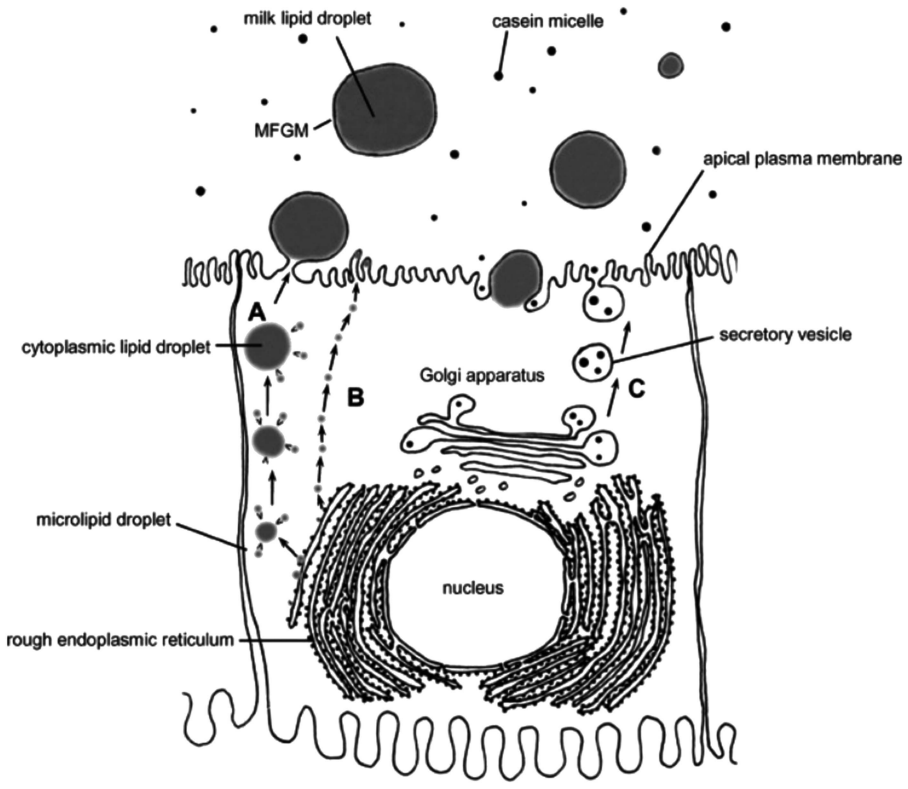


Figure 2 Milk fat secretion and synthesis by the mammary epithelial cell. A = cytoplasmatic lipid droplet pathway; B = microlipid droplet pathway; C = secretory pathway for skim milk components; MFGM = milk fat globule membrane (Bauman *et al.*, 2006).

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2017), even if it has been asserted that both SCC and lactose have to be monitored at the same time to enhance evaluation of udder health status (Haile-Mariam and Pryce, 2017).

The reason why there were no relationships between gelsolin and lactose and protein can be explained by the different secretion process (exocytosis) of the other major components of milk. Caseins, many whey proteins, lactose, water and ions accumulate in Golgi-derived secretory vesicles (Bauman *et al.*, 2006).

Conclusion

GSN expression analysis confirmed the link between gelsolin gene function and milk fat content of sheep. The identified variants in *GSN* together with the validation of the differential gene and protein expression may better explain the genotype–phenotype association. Moreover, it could be more profitable in marker selection for desired traits. This research broadened *GSN* knowledge, even if further studies are necessary to confirm our hypothesis in other livestock species.

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Declaration of interest

The authors report no conflicts of interest in this work.

Ethics statement

The trial was conducted in an experimental farm of the CREA following the recommendations of European Union directive 86/609/EEC.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119000399

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