# Effects of lactation stage, and milk SCC on whey protein composition in Sarda dairy ewes

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## ABSTRACT

In 90 Sarda dairy ewes the effects of lactation stage, parity, β-lactoglobulin genotypes, and somatic cell count (SCC) on the milk content of total protein (TP), casein (CN), whey protein (WP) and its fractions α-lactalbumin (ALA), β-lactoglobulin (BLG), serum albumin (SA), immunoglobulin (IG) and lactoferrin (LF) were analysed using a linear mixed model. Mean values of variables (g/l) were: TP (54.0), CN (43.0), WP (11.0), BLG (4.78), ALA (1.37), SA (0.61), IG (3.83) and LF (0.28). The lactation stage significantly affected all the variables analysed. TP, CN and WP concentrations tended to increase throughout lactation, with the increase of WP being more pronounced than the corresponding variation in CN. There was no definite trend in BLG content, whereas ALA concentration decreased as lactation progressed. The parity affected almost all variables studied. WP concentration differed significantly only between the second and fourth parity (10.45 vs 11.44 g/l). BLG and SA concentrations were significantly lower in the youngest ewes. The BLG genotype affected milk yield, but no effects were observed on the components of the milk. The SCC influenced almost all variables studied. The TP concentration was significantly higher in milk with SCC >1,000,000 (55.0 g/l) than in milk with lower SCC (53.4 g/l). This was mainly due to the increase of WP (12.52 and 10.24 g/l in milk with SCC above and below 1,000,000/ml respectively), especially in those WP fractions originating from blood.

*Key words:* Dairy ewes, Milk whey protein, Lactation stage, Parity, SCC

## RIASSUNTO

### EFFETTI DELLO STADIO DI LATTAZIONE, DELL'ORDINE DI PARTO, DEL GENOTIPO DELLA β-LATTOGLOBULINA E DEL CCS SULLA COMPOSIZIONE DELLE SIEROPROTEINE DEL LATTE IN PECORE DI RAZZA SARDA

*Su un gregge di 90 pecore di razza Sarda è stato studiato l'effetto dello stadio di lattazione, dell'ordine di parto, del genotipo al locus β-lattoglobulina e del contenuto in cellule somatiche (CCS) sui contenuti in proteina totale (TP), caseina (CN) e sieroproteina (WP) e sulle frazioni sieroproteiche β-lattoglobulina (BLG), α-lattoalbumina (ALA), sieroalbumina (SA), immunoglobuline (IG) e lattofferina (LF), con l'impiego di un modello lineare misto. I valori medi delle variabili esaminate (g/l), sono stati: TP (54,0), CN (43,0), WP (11,0), BLG (4,8), ALA (1,4), SA (0,61), IG (3,83) e LF (0,28). Lo stadio di lattazione ha influenzato significativamente tutte le variabili esaminate. I contenuti in TP, CN e WP sono tenden-*

*zialmente aumentati nel corso della lattazione con una aumento delle WP più pronunciato della corrispondente variazione in CN. Il contenuto in BLG non ha evidenziato un trend ben definito, mentre l'ALA è diminuita con il progredire della lattazione. Il contenuto in WP è risultato significativamente differente tra gli animali di secondo e quarto ordine di parto (10.5 vs 11.4 g/l). I contenuti in BLG e SA sono risultati più bassi negli animali più giovani. Nessuna influenza dell'ordine di parto è stato riscontrato sulle altre frazioni sieroproteiche. Il genotipo al locus BLG ha influenzato significativamente solo la produzione di latte, mentre nessun effetto è stato riscontrato su tutti i componenti del latte esaminati.*
*Il CCS ha influenzato significativamente quasi tutte le variabili analizzate. Il contenuto in TP è risultato significativamente più elevato nel latte con CCS >1,000,000 (55.0 g/l) rispetto al latte con un CCS inferiore (53.4 g/l). Ciò è dovuto principalmente ad un aumento delle WP (12.52 e 10.24 g/l nel latte con CCS maggiori e minori di 1.000.000/ml rispettivamente) ed in particolare di quelle di derivazione ematica.*

Parole chiave: *Pecore da latte, Sieroproteine, Stadio di lattazione, Ordine di parto, CCS*

## Introduction

Whey is the principal by-product of the dairy sheep industry, as almost all sheep milk is used to make cheese. Some 674,000 tons of sheep milk were produced in 1999 and 613,000 tons of this was processed into cheese (Pulina and Furesi, 2001). As a result, given an average cheese yield of 0.20, one can estimate that some 490,000 tons of whey were produced. Only a part of the whey is used to produce "Ricotta", a soft cheese cream obtained from heating the whey to 80°C. Most of it is thrown away and this creates serious waste disposal problems. Due to its chemical composition (c. 90% water, 1.25% fat, 1.63% protein and 4.75% lactose), sheep milk whey is a potential source of recyclable proteins (Comendador *et al.*, 1996). In recent years there has been increasing interest in the principal whey proteins (WP) such as β-lactoglobulin (BLG), α-lactalbumin (ALA), immunoglobulin (IG), serumalbumin (SA) and lactoferrin (LF). This is because they have biomedical, technological and functional properties, such as water holding, gelling, surface activity (Euston and Hirst, 2000). WP may have anti-carcinogenic (Hakkak *et al.*, 2000) and immuno-modulating effects (Cross and Gill, 1999), and is also used to produce bioactive peptides (Philanto-Leppala *et al.*, 2000). There is also a notable relationship between WP and the technical properties of milk; denaturation of BLG at high temperatures markedly reduces the rennetability of the milk (Dalgleish, 1993). Moreover, if the concentration of WP in milk is high this may reduce the drainage of curd and thus cause undesirable proteolysis or delay the maturing of the cheese (Mistry and Maubois, 1993).

The protein fraction contents in sheep milk are affected by environmental and genetic factors. Some research has been carried out on relationship between the genetic variants (A and B) of BLG and milk production, composition and coagulation properties (Giaccone *et al.*, 2000; Herget *et al.*, 1995; Rampilli *et al.*, 1997), but little information is available on the principal factors which influence the concentration of WP fractions.

Rampilli *et al.* (1997) in their study on Massese ewes reported higher BLG concentrations in BLG-AA genotype, while Duranti and Casoli (1991) found that WP content tends to increase as the SCC increases. More information on the main factors affecting the concentration and composition of WP in sheep milk is required for it to be correctly exploited.

The aim of the present work is to evaluate the effects of lactation stage, parity, BLG genotype and SCC on milk WP fractions in dairy ewes.

## Material and methods

A flock of 211 Sarda ewes was screened for BLG genotypes in order to create balanced BLG groups for parity order. Three groups of 30 ewes each were selected for each BLG genotype (AA, AB, and BB). Each group was composed of 10, 5, 10 and 5 ewes for 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ parity. Lambings took place over period of 10 days. Ewes were fed on natural (clover) and artificial (common oats, vetch) pasture, plus a supplement of 300-400 g/head of concentrate at each milking. All 90 selected ewes tested negative for the California Mastitis Test at the

Table 1. Arithmetic means and standard errors of milk yield, milk composition, whey protein fraction and SCC

| | | n. | Mean | SE |
|---|---|---|---|---|
| Milk | g/d | 495 | 1077 | 13.70 |
| Fat | g/l | 495 | 66.39 | 0.54 |
| Lactose | " | 495 | 43.90 | 0.29 |
| Total protein (TP) | " | 492 | 54.00 | 0.25 |
| - Casein (CN) | " | 492 | 42.98 | 0.22 |
| CN/TP | | 492 | 0.80 | 0.00 |
| Whey protein | " | 491 | 11.03 | 0.14 |
| - β-lactoglobulin | " | 491 | 4.78 | 0.08 |
| - α-lactalbumin | " | 491 | 1.37 | 0.02 |
| - Serum albumin | " | 491 | 0.61 | 0.02 |
| - Immunoglobulin | " | 491 | 3.83 | 0.09 |
| - Lactoferrin | " | 476 | 0.28 | 0.01 |
| SCC $10^3$ | $Log_{10}$ | 495 | 2.88 | 0.03 |

beginning of the trial. A mastitis control program was not applied during the experiment, but mastitis was detected visually in two ewes during the period of the experiment.

Individual milk yield was measured every two weeks from March to June at both daily milkings (morning and evening), beginning from 90 days in milking (DIM). Individual samples from morning and evening milking, weighted according to their respective milk yields, were analysed.

*Laboratory analyses*

Total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined by the Kjeldahl method, following the FIL-IDF methodology (1993; 1996). Casein content (CN) was calculated as (TN - NCN) x 6.38 and total whey protein (WP) as (NCN - NPN) x 6.38. Fat and lactose concentration was measured on a Milkoscan 605 (Foss Electric, Hillerød, Denmark) and SCC on a Fossomatic 360 (Foss Electric, Hillerød, Denmark).

BLG genotypes were determined by isoelectric focusing. Electrophoretic fractioning of the whey proteins was made by SDS-PAGE, as described by Feligini *et al.* (1997). Gels were automatically scanned with a GS-670 Densitometer (Bio-rad) to determine densitometric peak areas. The data are reported as an area proportion of each WP fraction and were converted to concentrations (g/l) of BLG, ALA, LF, SA, and IG, based on the WP concentration.

*Statistical analysis*

Data were analysed using the mixed linear model:

$$Y_{ijklpo} = \mu + DIM_i + P_j + G_k + SCC_l + (DIM \times SCC)_{il} + E_p + \varepsilon_{ijklpo}$$

where

- $Y$ = the dependent variable considered,
- $\mu$ = overall mean,
- $DIM_i$ = fixed effect of DIM i (i = 6 DIM of 2 week intervals each, starting from 90 DIM),
- $P_j$ = fixed effect of the parity j (j = 2,3,4,5 parity),
- $G_k$ = fixed effect of the BLG genotype k (k: AA; AB; BB),
- $SCC_l$ = fixed effect of somatic cells (l = 3 levels; SCC<500,000; SCC between 500,000 and 1,000,000; and SCC >1,000,000 cells/ml),
- $E_p$ = random effect of ewe p (p = 1…90),
- $\varepsilon_{ijkpo}$ = random residual.

The SCC classes were established following those suggested by Bergonier and Berthelot (2003), to distinguish between healthy and infected udders.

## Results and discussion

Means of milk yield, fat, lactose, TP, CN, WP and WP fractions and SCC are reported in table 1. Daily milk yield and SCC values were close to the mean for the Sarda breed (ARA, 2002). Fat, lactose, TP, CN and WP contents are within the range of values reported in other studies for Sarda (Pulina *et al.*, 1993) and other Mediterranean dairy sheep breeds (Cavani *et al.*, 1991; Fuertes *et al.*, 1998). BLG and ALA concentrations agree with previous results in dairy sheep (Duranti and Casoli, 1991; Law, 1995) but are higher than those reported for goats (Law, 1995) and cows (Law, 1995; Mackle *et al.*, 1999). As cow milk is the major industrial source of serum proteins, this result is particularly interesting because sheep milk may be a new and more profitable source of whey protein. The LF was higher than the values observed in sheep (Qian *et al.*, 1995), cow (Sanchez *et al.*, 1988) and goat milk (Greppi and Roncada, 2001). The IG and SA concentrations were also higher than those previous reported for sheep (Duranti and Casoli, 1991; Law, 1995), cows and goats (Law, 1995).

The SCC classes significantly influenced almost all variables considered, except fat and CN content (Table 2). The highest milk yield has found in milk with SCC<1,000,000. The CN content tended to decrease as the SCC rose, while the WP and its fractions were significantly lower in the milk with SCC<1,000,000. An increase in WP fractions is usually evidence of mammary gland inflammation (Sordillo *et al.*, 1997; Urech *et al.*, 1999). The high content of IG and SA in mammary secretion derives mostly by paracellular passage through disrupted tight junctions, or by enhanced specific IG receptors during involution or intramammary inflammation (Schanbacher *et al.*, 1997). Milk LF comes from the degranulation of blood neutrophils (Neville and Zhang, 2000; Sordillo *et al.*, 1997), which were the only type of somatic cells that increased in sheep milk as the SCC rose (Cuccuru *et al.*, 1997), and from mammary cell synthesis (Sanchez *et al.*, 1992; Schanbacher *et al.*, 1997). Although mastitis was detected by visual inspection in only two ewes during the experiment, about 50% of the ewes could be considered as potentially infected according to Bergonier and Berthelot's rule (2003). Thus a possible explanation for the increase of SA and IG in the highest SCC class could be the higher supply of these proteins from blood; it cold also be partly caused by increased concentration, due to the lower milk yield with SCC over 1,000,000. The increase of BLG and ALA concentration in milk with SCC>1,000,000, should be mainly attributed to concentration effect rather than to an increase of syn-

Table 2. Least square means of milk yield, milk composition and whey protein fraction in milk of animals classified according to the SCC

| | | SCC classes | | |
|---|---|---|---|---|
| | | <500,000 | 500,000-1,000,000 | >1,000,000 |
| Milk | g/d | 1114[a] | 1104[a] | 1015[b] |
| Fat | g/l | 66.15 | 67.07 | 68.04 |
| Lactose | " | 45.33[A] | 44.42[A] | 42.35[B] |
| Total protein (TP) | " | 53.57[a] | 53.17[a] | 55.05[b] |
| - Casein (CN) | " | 43.59 | 42.70 | 42.54 |
| CN/TP | | 0.81[A] | 0.80[A] | 0.77[B] |
| Whey protein | " | 10.07[A] | 10.41[A] | 12.52[B] |
| - β-lactoglobulin | " | 4.60[A] | 4.62[A] | 5.29[B] |
| - α-lactalbumin | " | 1.28[a] | 1.34[a] | 1.47[b] |
| - Serum albumin | " | 0.55[a] | 0.60[a] | 0.72[b] |
| - Immunoglobulin | " | 3.30[A] | 3.44[A] | 4.44[B] |
| - Lactoferrin | " | 0.20[A] | 0.23[A] | 0.41[B] |

*[a,b]P<0.05; [A,B]P<0.01*

Figure 1. Time evolution of milk yield, fat, total protein, and lactose in milk with different SCC classes (<500,000; 500,000-1,000,000; >1,000,000 cells/ml)

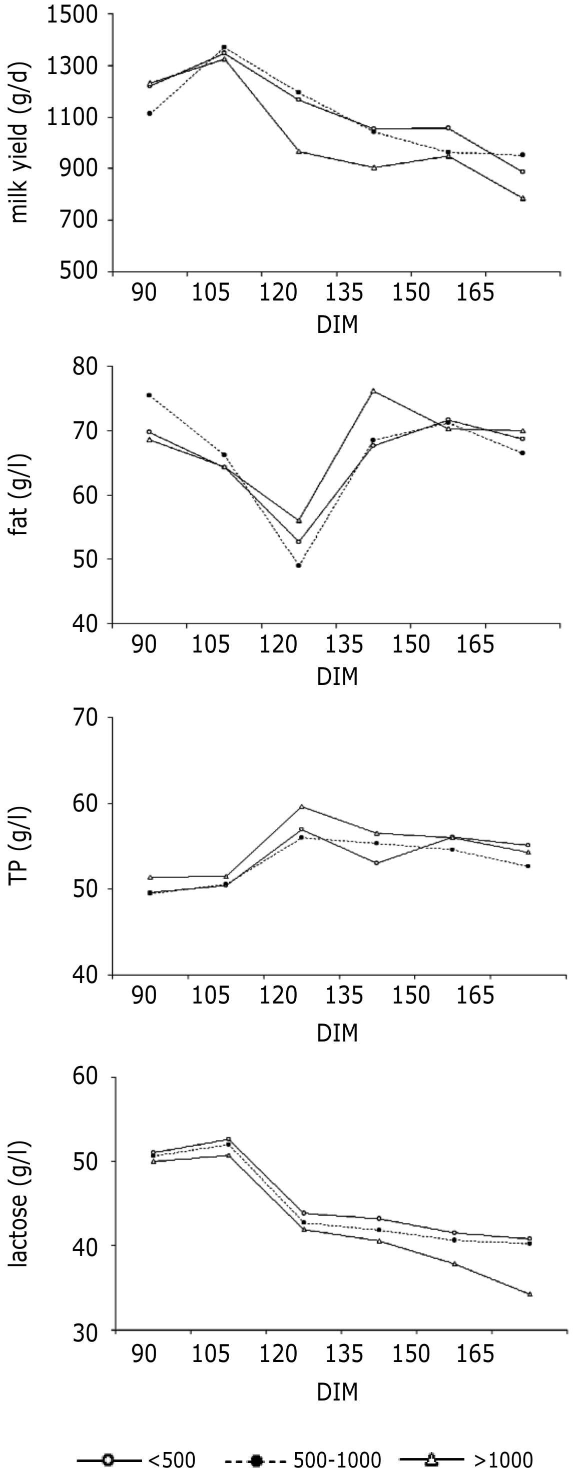
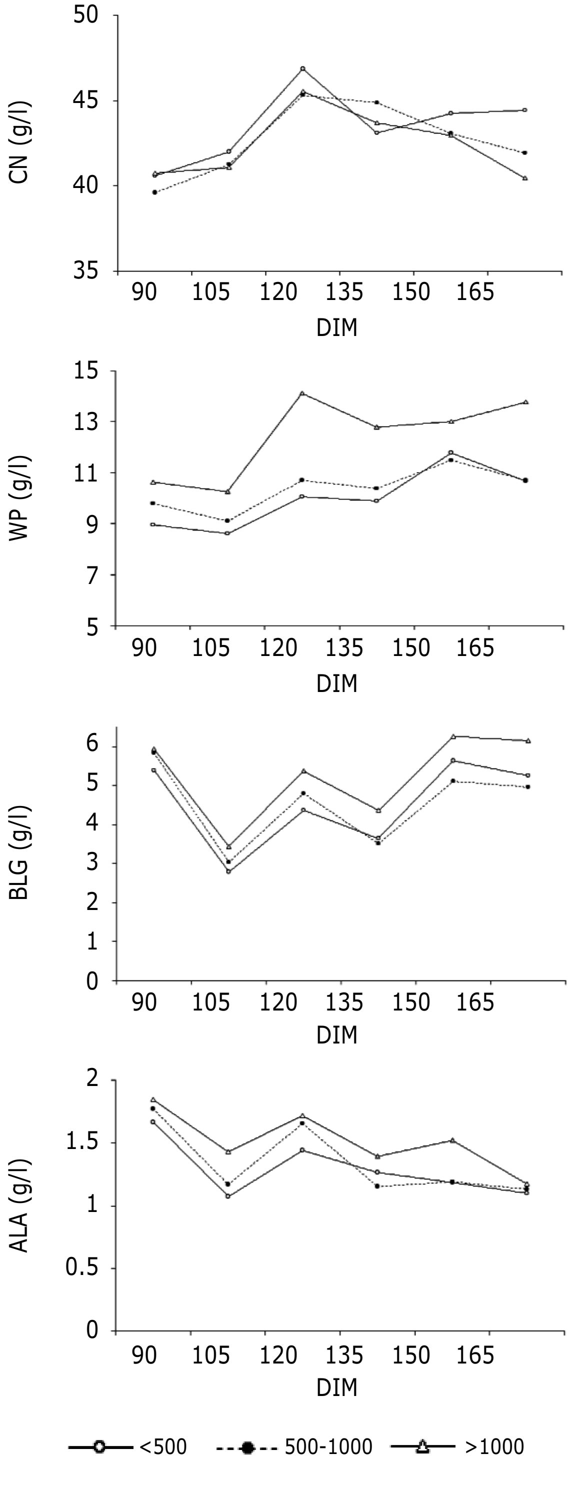


Figure 2. Time evolution of casein, total whey protein and whey protein fractions synthesised by the mammary gland in milk with different SCC classes (<500,000; 500,000-1,000,000; >1,000,000 cells/ml)



thesis of these proteins by the mammary cells: there is an increase of BLG and ALA of about 13% in the SCC>1,000,000 class, which corresponds with a reduction of milk yield of the same extent (Table 1).

DIM affected all variables studied significantly, as was expected. The patterns of the variables under consideration during lactation, separated for different SCC classes, are reported in Figure 1, 2 and 3. Milk yield (Figure 1) decreased from 105 DIM towards the end of the experiment, especially in the higher SCC class. The decrease of fat content at 120 DIM can be explained by phenomenon of subacidosis due to the high doses of concentrate in the diet and the lower NDF content of the grass in spring. The TP content increased to 120 DIM due to effects of concentration, and remained fairly stable until the end of the experiment. Lactose concentration decrease during lactation, following the pattern of milk yield, and was significantly lower in class with higher SCC only at 150 and 165 DIM. The CN content (Figure 2) showed a pattern similar to TP content in all SCC classes except at 165 DIM, where a significant difference was found between the highest and the lowest SCC class. The WP content tended to increase during lactation, but this was more marked in the highest SCC class after 120 DIM.

There was no definite trend in BLG content, although some authors have reported an increase during lactation both in sheep (Bolla *et al.*, 1989) and goats (Quiles *et al.*, 1994). The ALA concentration tended to decrease during lactation, as did milk yield and lactose content. Similar behaviour for milk yield, lactose and ALA concentration have been already observed in dairy cattle, where the reduction in milk yield was attributed to the diminished synthesis of lactose caused by the reduction in ALA concentration (Prosser and McLaren, 1997).

The SA and IG content increased, peaking at 120 and 135 DIM, respectively (Figure 3). LF content remained fairly stable for the whole experimental period in milk with SCC<1,000,000. It was, however, markedly higher in the highest SCC class, and decreased from 120 DIM to the end of experiment.

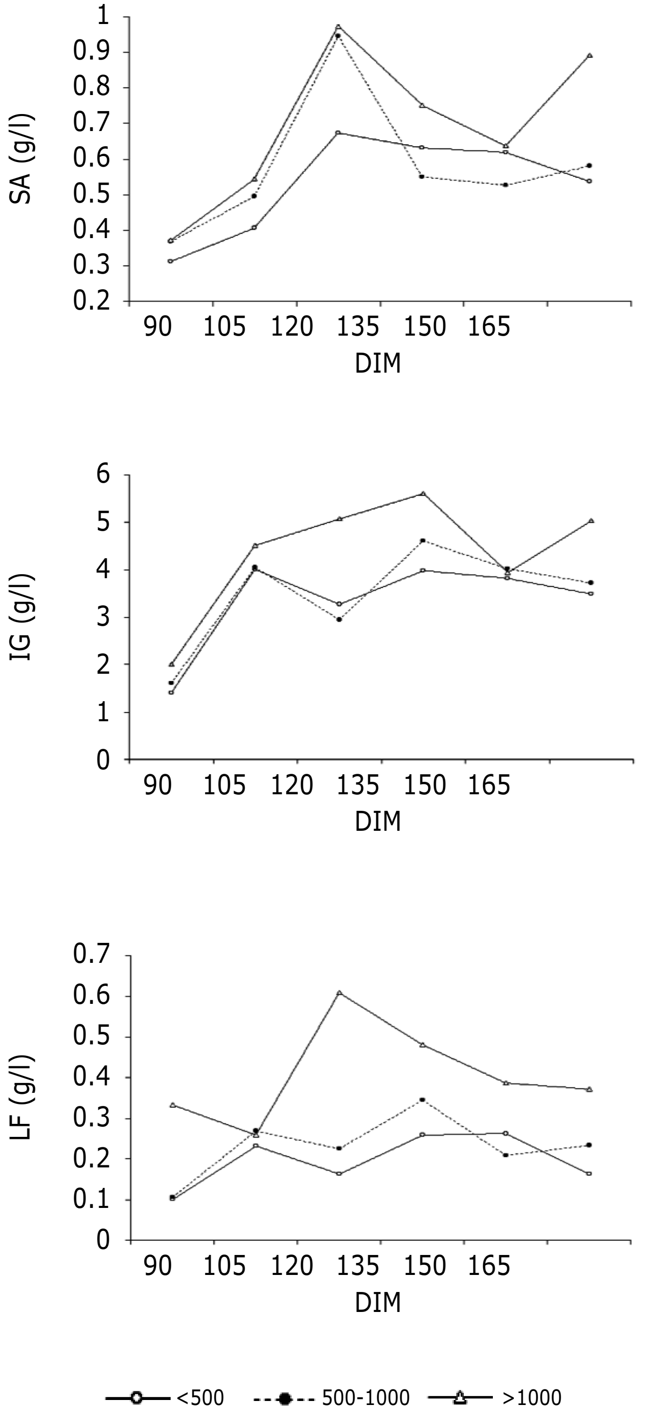
The parity influenced almost all the variables considered significantly, except CN/TP, ALA, IG and SCC (Table 3). TP, CN and WP tended to increase with parity, reaching the highest value at fourth parity. An increase of these protein with age has been reported for the Churra breed (Fuertes *et al.*, 1998) whereas Casoli *et al.* (1989) reported a decrease of total WP concentration in the Massese breed with parity. BLG and SA concentrations were lowest in the youngest ewes, while no difference was observed

Table 3. Least square means of milk yield, milk composition, whey protein fraction and SCC in milk with different parities

| | | Parity | | | |
|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 |
| Milk | g/d | $1164^{a}$ | $1129^{a}$ | $1042^{b}$ | $975^{b}$ |
| Fat | g/l | $62.68^{A}$ | $68.50^{B}$ | $69.75^{B}$ | $67.40^{B}$ |
| Lactose | " | $44.70^{A}$ | $43.14^{B}$ | $44.21^{AB}$ | $44.08^{AB}$ |
| Total protein (TP) | " | $52.20^{a}$ | $54.22^{b}$ | $55.31^{b}$ | $54.01^{b}$ |
| - Casein (CN) | " | $41.75^{a}$ | $42.92^{ab}$ | $43.88^{b}$ | $43.21^{b}$ |
| CN/TP | | 0.80 | 0.79 | 0.79 | 0.80 |
| Whey protein | " | $10.45^{a}$ | $11.38^{ab}$ | $11.44^{b}$ | $10.72^{ab}$ |
| - β-lactoglobulin | " | $4.32^{a}$ | $5.20^{b}$ | $5.04^{b}$ | $4.77^{b}$ |
| - α-lactalbumin | " | 1.41 | 1.41 | 1.36 | 1.28 |
| - Serum albumin | " | $0.51^{A}$ | $0.67^{B}$ | $0.66^{B}$ | $0.66^{B}$ |
| - Immunoglobulin | " | 3.78 | 3.63 | 3.90 | 3.59 |
| - Lactoferrin | " | $0.25^{a}$ | $0.29^{ab}$ | $0.33^{b}$ | $0.24^{a}$ |
| SCC $10^{3}$ | $Log_{10}$ | 2.90 | 2.90 | 2.90 | 2.84 |

$^{a,b}P<0.05$; $^{A,B}P<0.01$

Figure 3. Time evolution of whey protein deriving from blood in milk with different SCC classes (<500,000; 500,000-1,000,000; >1,000,000 cells/ml)



among other parities. This agrees with previous results obtained for the Sarda breed (Bolla *et al.*, 1989). The lower concentration in SA in second parity ewes can be explained by the better physiological condition of the udder in young animals, as is confirmed by the higher lactose concentration and milk yield. The SCC did not differ significantly among parities. This is different from previous results, where an increase of the SCC with number of lactations was reported in sheep (Bergonier *et al.*, 1996; Bencini and Pulina, 1997).

BLG genotype (Table 4) had a significant effect only on milk yield. BLG BB ewes had the lowest milk yield. No effects were observed on milk composition.

However studies on the relationships between BLG genotype and composition have produced conflicting results. Some authors found that the BLG genotype affects TP, CN and WP (Di Stasio *et al.*, 1997; Giaccone *et al.*, 1997; Herget *et al.*, 1995) whereas others did not (Barillet *et al.*, 1993; Taibi *et al.*, 1999). These differences, which also occur in studies on dairy cattle, may be due to the structure of the data analysed and the model used for statistical analysis (Giaccone *et al.*, 2000; Ng-Kway-Hang, 1997). The balanced experimental design and the mixed linear model used in this study allowed the BLG genotype classes to be compared more accurately, thus avoiding the lack of balance among genotypes, which is one of the most conspicuous features of the studies cited above.

## Conclusions

Our results confirm that sheep milk has higher WP content than cow or goat milk. WP content and its fractions varied mainly according to lactation stage and milk SCC. The increase of TP in milk during lactation and in milk with SCC above 1,000,000 was principally due to the increase of WP, particularly for those fractions which originated from blood. Thus milk produced in late lactation can be considered as an important source of useful whey proteins.

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Table 4. Least square means of milk yield, milk composition, whey protein fraction and SCC in milk with different β-lactoglobulin genotypes

| | | βLG Genotype | | |
|---|---|---|---|---|
| | | AA | AB | BB |
| Milk | g/d | 1116[A] | 1129[A] | 988[B] |
| Fat | g/l | 67.37 | 67.64 | 66.24 |
| Lactose | " | 44.38 | 43.76 | 43.97 |
| Total protein (TP) | " | 53.47 | 53.85 | 54.48 |
| - Casein (CN) | " | 42.68 | 42.57 | 43.57 |
| CN/TP | | 0.80 | 0.79 | 0.80 |
| Whey protein | " | 10.79 | 11.31 | 10.89 |
| - β-lactoglobulin | " | 4.82 | 4.91 | 4.77 |
| - α-lactalbumin | " | 1.34 | 1.40 | 1.35 |
| - Serum albumin | " | 0.62 | 0.66 | 0.60 |
| - Immunoglobulin | " | 3.56 | 3.89 | 3.74 |
| - Lactoferrin | " | 0.28 | 0.28 | 0.26 |
| SCC $10^3$ | $Log_{10}$ | 2.90 | 2.85 | 2.90 |

A,B *P<0.01*

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