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Full Length Research Paper

Effects of somatic cell count on the gross composition, protein fractions and mineral content of individual ewe's milk

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The aim of the research was to evaluate the effect of two somatic cell content (SCC) levels (<265,000 and >265,000 cells/ml) on ewe milk composition, protein fractions and mineral content. Samples were collected during two years, from three different ewe herds. Each herd was surveyed four times per year, one per season. For each survey more than 10 individual milk samples were collected during morning milking and analysed for SCC. On the basis of the results, two individual milk samples were selected: one from a sheep with low milk SCC (up to 265.000 cells/mL; LCC) and one from a sheep with high milk SCC (over 265,000 and less than 1,000,000 cells/mL; HCC). In one herd, it was not possible to collect the milk samples in summer. So, a total of 44 ewe milk samples (22 comparative pairs) were collected. On each milk sample, crude protein, crude whey protein, casein, casein number, non protein nitrogen x 6.38, true protein, true whey protein, fat, lactose, dry matter, ash, phosphorus, calcium, magnesium and chloride were determined. Average SCC in LCC was 170,000 and 466,000 cells/ml in HCC milk. HCC milk had lower lactose (4.05 vs. 4.60 g/100 g), casein (3.91 vs. 4.28 g/100 g), phosphorus (131.31 vs. 138.81 mg/100 g), calcium (157.28 vs. 170.48 mg/100 g) and magnesium (14.59 vs. 15.30 mg/100 g) contents than LCC milk. Additionally, HCC milk had lower casein number (76.53 vs. 79.03%) and higher contents of true whey protein (1.00 vs. 0.92 g/100 g), ash (0.90 vs. 0.87 g/100 g) and chloride (103.57 vs. 93.17 mg/100 g) than LCC milk. Somatic cell content significantly affected ewe milk quality. As a result of the higher lactose, casein, phosphorus, calcium and magnesium contents, LCC milk was more suitable for cheese making than HCC milk.

Key words: Ewe milk, milk quality, chemical composition, somatic cell content.

INTRODUCTION

The mammary gland inflammation process is characterised by an increased number of somatic cells (mainly leucocytes) in milk (Viguier et al., 2009; Le Maréchal et al., 2011). The inflammatory response is also characterised by a transfer of some blood components in the milk (Urech et al., 1999) and by a decrease in the secretory activity, resulting in a reduced milk production (Gonzalo et al., 2002; Le Maréchal et al., 2011). Moreover, the milk secreted is characterised by some alterations of chemical composition and physico-chemical properties (Bianchi et al., 2004; Raynal-Ljutovac et al., 2007; Le Maréchal et al., 2011). These changes in milk composition are associated with flavour defects in yogurt (Vivar-Quintana et al., 2006) and flavour and texture alterations in the cheese (Le Maréchal et al., 2011).

Currently, Italy does not implement any program aimed to reduce somatic cell content (SCC) in ewe milk; consequently, the average content of SCC in ewe milk in

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Italy is high. Castagnetti et al. (2000) reported that average SCC of ewe bulk milk in Emilia Romagna region (Northern Italy) was 1,728,000 cell/ml and Rosati et al. (2005), in a study carried out on up to 2,000 herd milk samples, reported that the average SCC of ewe bulk milk in Italy was 1,389,000 cells/ml. According to Rosati et al. (2005), the SCC of individual ewe milk above the threshold value of 265,000 cells/ml is indicative that the ewe is affected by mastitis; however, in their study, these authors did not consider the possible effects on the qualitative composition of the milk.

In the literature, there are few papers that examine the effects of somatic cells on the properties of raw ewe milk; some studies considered pools of individual milks or herd milks, and only limited studies were carried out at individual milks level. Moreover, the effects of somatic cells on mineral content have been rarely studied and no paper was published considering some minerals such as magnesium (Le Maréchal et al., 2011). Furthermore, the few studies available in the literature considered a value lower than 500,000 cells for normal milks, and values over a million for milks with "high content".

Changes in ewe milk composition markedly affect the quality and the characteristics of dairy products (Coulon et al., 2004; Vivar-Quintana et al., 2006; Le Maréchal et al., 2011); for this reason it is important to evaluate the influence of factors affecting milk composition, as somatic cells.

For all these aspects considered above, the aim of this research was to evaluate the effect of two levels of SCC (<265,000 and >265,000 cells/mL) on ewe milk composition, protein fractions and mineral content.

MATERIALS AND METHODS

Samples were collected during two years, from three different ewe herds, located in Parma and Reggio Emilia province (Emilia Romagna region, North of Italy). Herds were similar in size and management practices, and representative of the typical ewe herd of the region. Herds were surveyed four times each year, one per season (winter from January to March, spring from April to June, summer from July to September, autumn from October to December). In each survey, about 10 individual milk samples were collected from as many sheep that were homogeneous for breed, weight, stage of lactation and parities. The ewe milk samples (500 ml) were taken during morning milking, preserved with sodium merthiolate (0.02% w/v) ($C_9H_9HgNaO_2S$; Carlo Erba Reagents 20090, Milano, Italy), immediately cooled at 5°C, transported to laboratory and submitted to analysis for SCC content. The analysis was performed by fluoro-opto-electronic method (Schmidt-Madsen, 1975), with Fossomatic 250 (Foss Electric, DK-3400 Hillerød Denmark). Then, on the basis of the results, from the ten screened sheep, a pair of sheep was selected, one sheep with low milk SCC (up to 265,000 cells/ml; LCC) and the other with high milk SCC (over 265,000 and less than 1,000,000 cells/ml; HCC). In one herd, it was not possible to collect the samples during summer. So, a total of 44 ewe milk samples (22 comparative pairs) were involved.

On each milk sample, the following parameters were analysed: 1) nitrogen fractions: total nitrogen (TN), soluble nitrogen (SN) and non protein nitrogen (NPN) by Kjeldahl, respectively, on whole milk, on acid whey at pH 4.6 and on filtered 12% TCA (Aschaffenburg and Drewry, 1959); the following parameters were then calculated: crude protein (TNx6.38/1000), crude whey protein (SNx6.38/1000), casein ((TN-SN)x6.38/1000), casein number ((TN-SN)x100/TN), NPNx6.38 (NPNx6.38/1000), true protein ((TN-NPN)x6.38/1000) and true whey protein ((SN-NPN)x6.38/1000); 2) fat and lactose contents, determined by mid-infrared spectrophotometer analysis (Biggs, 1978), with Milko-Scan 134 A/B (Foss Electric, DK-3400 Hillerød Denmark); 3) dry matter and ash were obtained, respectively, after oven drying at 102°C and muffle furnace calcination at 530°C (Savini, 1946); the ash was diluted with 2N HCl for the determination of phosphorus, by means of the colorimetric method of Allen (1940), and calcium and magnesium contents, by means of atomic absorption spectrophotometry (De Man, 1962); 4) chloride (Cl^-) by titration with silver nitrate using the volumetric method of Charpentier-Volhard (Savini, 1946).

Data were submitted to ANOVA (SPSS ver. 18.0, Chicago, IL 60606, USA) using the SCC classes (2 levels, LCC or HCC) and season (4 levels; winter, spring, summer and autumn) as fixed effect and covariate for the parity and day in milk effects. The significance of the differences was tested by means of least significant difference (LSD) control. Finally, data were processed by the Pearson product moment correlation coefficient to measure the degree of linear correlation between somatic cells (expressed as logarithm), casein content and the other main milk chemical composition parameters.

RESULTS

The average value of somatic cell content was 170,000 cells/ml (standard deviation ±39,000) with a minimum of 101,000 and a maximum of 236,000 cells/ml in the LCC milk and 466,000 cells/ml (standard deviation ±201,000) with a minimum of 270,000 and a maximum of 839,000 cells/ml in the HCC milk (data not shown).

The main chemical composition parameters, protein fractions and mineral content of the individual ewe's milk samples with low cell content (LCC) and high cell content (HCC) are shown in Table 1. The HCC milk had lower contents of lactose (4.05 vs. 4.60 g/100 g; P≤0.01) and casein (3.91 vs. 4.28 g/100 g; P≤0.05). The HCC milk also had higher true whey protein content (1.00 vs. 0.92 g/100 g; P≤0.05). The decrease in the casein content, accompanied by an increase in the whey protein, leads to a variation in the casein number; in fact, HCC milk show a markedly lower casein number as compared to LCC milk (76.23 vs. 79.11%; P≤0.001).

HCC milk had higher ash (0.90 vs. 0.87 g/100 g; P≤0.05) and chloride (103.57 vs. 93.17 mg/100 g; P≤0.05) contents than LCC milk. On the other hand, HCC milk had lower phosphorus (131.31 vs. 138.81 mg/100 g; P≤0.05), calcium (157.28 vs. 170.48 mg/100 g; P≤0.05) and magnesium (14.59 vs. 15.30 mg/100 g; P≤0.05) contents than LCC milk.

Pearson product moment correlation coefficients between the milk SCC (expressed as logarithm), casein content and the other main milk chemical composition parameters are shown in Table 2. SCC showed positive correlations (P≤0.05) with whey protein (r = 0.197), chloride (r = 0.201) and ash (r = 0.148) contents, while it

Table 1. Least square mean value ± standard deviation of milk composition, protein fractions and mineral content of the individual ewe's milk samples with low cell content (LCC) and high cell content (HCC).

Parameter	LCC (n[1]=22)	HCC (n[1]=22)	*P*[2]
Dry matter (g/100 g)	19.15±2.48	17.80±2.90	NS
Lactose (g/100 g)	4.60±0.57	4.05±0.98	**
Fat IR (g/100 g)	8.29±2.07	8.00±2.01	NS
Crude protein (g/100 g)	5.41±0.94	5.13±0.86	NS
Crude whey protein (g/100 g)	1.13±0.18	1.22±0.26	*
Casein (g/100 g)	4.28±0.80	3.91±0.70	*
Casein number %	79.11±2.23	76.23±3.53	***
NPNx6.38 (g/100 g)	0.21±0.05	0.22±0.05	NS
True protein (g/100 g)	5.20±0.95	4.91±0.83	NS
True whey protein (g/100 g)	0.92±0.19	1.00±0.32	*
Ash (g/100 g)	0.87±0.08	0.90±0.07	*
Phosphorus (mg/100 g)	138.8±24.72	131.31±30.80	*
Calcium (Ca^{2+}) (mg/100 g)	170.48±27.48	157.28±18.64	*
Magnesium (Mg^{2+}) (mg/100 g)	15.30±3.71	14.59±2.79	*
Chloride (Cl^{-}) (mg/100 g)	93.17±10.46	103.57±25.25	*

[1]Number of individual ewe milk samples; [2]Significance of differences: NS, *P*>0.05; **P*≤0.05; ***P*≤0.01; ****P*≤0.001. Values are mean±SD.

Table 2. Pearson product moment correlation coefficients between milk somatic cells content (expressed as logarithm), casein content and other milk chemical composition parameters.

Parameter	Somatic cell		Casein	
	r	*P*[1]	r	*P*[1]
Casein	-0.395	*	_	_
Whey protein	0.197	*	0.704	**
Phosphorus	-0.417	*	0.537	**
Calcium (Ca^{2+})	-0.452	*	0.485	**
Magnesium (Mg^{2+})	-0.301	*	0.656	***
Chloride (Cl^{-})	0.201	*	0.026	NS
Ash	0.148	*	0.548	*

[1]Significance of differences: NS, *P*>0.05; **P*≤0.05; ***P*≤0.01; ****P*≤0.001.

showed negative correlations (P≤0.05) with casein (r = -0.395), phosphorus (r = -0.417), calcium (r = -0.452) and magnesium (r=-0.301) contents. Casein resulted positively correlated (P≤0.01) with whey protein (r = 0.704), phosphorus (r = 0.537) and calcium (r = 0.485) contents. Casein also showed a positive correlation with magnesium (r = 0.656; P≤0.001) and with ash (r = 0.548; P≤0.05).

DISCUSSION

Le Maréchal et al. (2011), in a recent review, reported that, in general, there is an increase in the concentration of protein compounds associated with the inflammatory and immune response and a decrease in endogenous milk protein such as casein; however, in the review, the threshold value of SCC for which presence of inflammation can be considered was not reported.

Other researchers evaluated the possible effects of the contents of SCC on milk quality (Jaeggi et al., 2003; Nudda et al., 2003; Albenzio et al., 2004; Bianchi et al., 2004; Leitner et al., 2004; Revilla et al., 2007), but it is important to underline that these authors have evaluated the effects of SCC and it was higher with respect to that we have considered, and not always on individual milks.

In particular, Nudda et al. (2003) and Bianchi et al. (2004) studied the effects of SCC on milk quality considering individual milks; Bianchi et al. (2004) considered udder as "healthy" if every month control of

SCC was below 500,000 during six months of lactation and as "infected" when in this period, there were at least two milk sampling with values above 1,000,000 cells. Nudda et al. (2003) grouped the samples into three classes of cells: less than 500,000 cells, between 500,000 and 1,000,000 cells and over 1,000,000 cells.

In dairy sheep, Leitner et al. (2004) compared milk from udder half infected and milk from the contralateral gland free of bacteria; the uninfected udder half had milk with a mean SCC of 311,000 cells, while the infected udder half milk had a mean SCC of 4,999,000 cells. Pirisi et al. (2000), Jaeggi et al. (2003), Albenzio et al. (2004) and Revilla et al. (2007) analysed pools of individual milks or herd milks. Pirisi et al. (2000) reported three groups of SCC: under 500,000, between 500,000 and 1,000,000 and over 1,000,000. Jaeggi et al. (2003) considered three groups: under 100,000 cells, between 100,000 and 1,000,000, and more than 1,000,000; moreover, milk was pasteurised before being analysed. Albenzio et al. (2004) reported two groups, one under 500,000 and the other over 1,000,000 cells. While Revilla et al. (2007) considered three groups, one under 500,000 cells, one between 1,000,000 and 1,500,000 and the other over 2,500,000 cells. The results of the present research are not consistent with data reported by these authors.

Nudda et al. (2003), Albenzio et al. (2004) and Bianchi et al. (2004) reported that an increase in SCC in ewe milk from less than 500,000 to over 1,000,000 cells was related to an increase in whey protein content and a decrease in lactose content. Revilla et al. (2007) reported a drop of lactose over 2,500,000 cells. Leitner et al. (2004) reported a lactose content decrease for infected milk; moreover, they observed that an increase in the SCC is correlated with a greater potential for proteolysis and, consequently with a decrease of casein content and casein number.

Jaeggi et al. (2003) found a decrease in casein corresponding to an increase in cells over 1,000,000. Moreover, in their research, they also showed a decrease in dry matter, a fact that was not observed at the SCC level studied in the present research; therefore, Jaeggi et al. (2003) did not see variations for either casein or dry matter when cells were between 100,000 and 1,000,000. Jaeggi et al. (2003) proposed that the increased proteolic activity, associated with the highest content of SCC, was due to proteolytic enzymes, such as plasmin or other proteases derived from somatic cells, which led to a break of the casein and, therefore to a decrease of casein content.

In their study, they suggest that there was not only a proteolysis of casein but also a decrease of its synthesis, as evidenced by the decrease of dry matter. In the present research, however, it was not possible to exclude this, because the decrease of casein synthesis could be counterbalanced by the increase of whey protein of haematic origin; and this aspect could explain the results reported in the present research, where both dry matter and also crude protein do not vary in a statistically significant way. However, in this research, we observed changes at a lower level of SCC, for which probably the damages to the mammary tissue could not be so marked.

Le Maréchal et al. (2011) reported that the effects of the increase of SCC in ewe milk on mineral content are not clear, but, also in this case, they did not refer to specific values of cells. The value of ash content reported here are in agreement with Bianchi et al. (2004) values for the milk with SCC of over 1,000,000. Unlike what was observed in the present study, both Pirisi et al. (2000) and Bianchi et al. (2004) did not observe variations of phosphorus content related to the increase of SCC; Bianchi et al. (2004) did not report variations also for chloride content. Concerning calcium content, different from what was observed in this research, Pirisi et al. (2000) did not observe variations related to the increase of SCC, while Bianchi et al. (2004) observed an increase of calcium content. The higher phosphorus, calcium and magnesium contents of LCC milk, with respect to HCC milk, observed in the present research are probably mainly due to the higher casein content.

Furthermore, Le Maréchal et al. (2011) reported that there are no data in the literature with regards to the effect of ewe milk SCC on magnesium contents.

Conclusions

Somatic cell content significantly affects ewe milk quality. Because of the higher lactose, casein, phosphorus, calcium and magnesium contents, milk with less than 265,000 cells/ml showed a higher quality than milk with more than 265,000 cells/ml and, therefore, it is more suitable for cheesemaking. On the other hand, the higher content of SCC in the HCC milk is responsible for the higher whey protein, ash and chloride contents and the lower lactose content of HCC milk.

This research suggests that there could be a possible alteration of milk quality also under 500,000 cells/ml. These experimental results, finally, together with data present in the literature, demonstrated that it is necessary to implement a program aimed to reduce the milk somatic cell count in ewe's milk, with the aim of improving the quality of ewe milk and dairy products.

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