Robotic milking systems (RMS) modify dairy herd management and therefore some aspects of production. The main results from scientific literature on RMS and cheese-making properties have been reported. The decrease in fat content, as a consequence of the increased milking frequency, is generally confirmed. The lack of specific studies on creaming properties of milk from robotic milking experiences and with different milking frequencies has been highlighted. Indications on clotting features were obtained with a different milking frequency in a traditional milking parlour; these results showed an improvement in the casein index of milk from three daily milkings. A reduction of casein exposure to the plasminogen-plasmin complex activity in the mammary gland between two consecutive milkings seems to explain this result. The effect of RMS on milk quality for cheese-making purposes was first evaluated in a two-year monitoring study in a herd representative of Po Valley dairy farms. Preliminary results from laboratory tests on bulk milk samples indicate that milk from RMS seems suitable for cheese-making processes.

Key words: Dairy cow, Robotic milking, Milk quality, Creaming properties, Clotting features

Introduction

In dairy cattle breeding, there is an increasing worldwide interest in the adoption of robotic milking systems (RMS). The diffusion of such systems currently seems to be limited mainly by economic evaluation in the interested countries. This system appears particularly suitable for medium-sized herds, where the farmer and his family are the main source of labour. The RMS also suits many farms of those countries where labour costs are very high and there is little propensity among young people to work as stockmen. Beside aspects related to milk-processing regulations, those for cheese manufacturing typical products are to be considered. As regards those regulations, it is necessary to understand the real effect of robotic milking on properties that may affect the cheese-making processes of typical products like Grana type cheeses.

Several kinds of factor may affect milk quality in an RMS when compared to the traditional
twice-daily (2×) milking system. First, the animal’s milk secretion could be affected by increased and irregular milking frequency; second, there could be the effect of transport to the tank and successive storage systems (Puhan, 1989), which differs between traditional and robotic systems.

Even if we do not specifically deal with the milk yield, we must take into account the effect that the different milking frequency may exert on the physiology of mammary tissues during milk secretion.

Recently, Boutinaud et al. (2003) confirmed with goats that milking frequency clearly affected epithelial cell numbers and alveolar diameter, with the result that the reduction of milking frequency induced a decrease in alveolar diameters. We can suppose that, if cistern volume is not affected, the role of the increased alveoli volume may affect milk fat content and composition. In fact, Friggens and Rasmussen (2001) justified their results in the light of works by Knight et al. (1994) and Dewhurst and Knight (1994), who showed that the distribution of milk within the udder was directly related to the time interval since the last milking. The shorter the interval since last milking, the greater the proportion of milk from the alveoli as opposed to the udder cistern. Alveolar milk has been found to have a substantially higher content of milk fat than cisternal milk (Davis et al., 1998); the differences in milk protein and lactose are much smaller. These findings indicate a strong biological basis for relationships between milk composition and the temporal distribution of milkings.

We might consider the effects on milk fat and protein separately because of the different effects on cheese production.

Fat percentage and quality affect creaming properties of the milk. This is an important issue if we consider that natural creaming is a preliminary stage of milk processing for cheese production such that of Grana.

Protein percentage and quality affect clotting features. The consequences of different patterns of clotting features affect cheese yield and quality.

In a previous paper (Abeni et al., 2002), we described milk quality parameters related to cheese-making properties during the transition from a traditional milking parlour system to a RMS. The present paper also reports data on bulk milk quality with RMS during the first two years of our experience. These data were obtained with a relatively constant diet (with corn silage, fescue hay and alfalfa hay as forage base) and with a rolling herd with an average of 40% of primiparous cows.

Figure 1. Least square means of the average daily milking frequency of the herd by season in the first two years of RMS. Bar without common letter indicates that seasons differ with $P < 0.05$. 

![Figure 1](image-url)
The modification in milking timing as a consequence of RMS introduction

The RMS is not a thrice-daily (3×) milking system. In fact, daily milking frequency can be varied from one (1×) to four or more milkings per day. Data from Hogeveen et al. (2001) on 107,785 milkings reported an average milking frequency of 2.6 milkings per day and an average milking interval of 9.2 h. Hogeveen et al. (2001) also recorded that 17.6 % of the milkings had a preceding milking interval of more than 12 h and 9.7 % of the milkings had a preceding milking interval shorter than 6 h. Our previous results reported that 19.0 % of the milkings had a preceding interval of more than 12 h and 12.5 % of milkings had a preceding interval shorter than 6 h (Speroni et al., 2003).

A prolonged interval between milkings may affect milk yield and composition (Vetharaniam et al., 2003). In our work, we needed to show whether season can act on RMS milk quality through variations in milking intervals. However, further research will be necessary to distinguish the effect of RMS by milking interval independently from the effect of the season (climate) per se. Throughout 2 yrs of robotic milking, we recorded a mean of 2.56 ± 0.21 milkings/cow/day. In Figure 1 the reduction in milking frequency during the summer is shown; this reduction derives mainly from the increase in the number of milkings with a preceding milking interval higher than 12 hours, as shown in Figure 2. Considering that the longer the milking interval, the greater the quantity milk from the milking is, it can be argued that milk characteristics from milking with increasing milking intervals during summer are important, because they can considerably influence cheese production.

The milk fat and its technological features

Milk fat content.

Milk fat content is affected by milking frequency, which could be an important source of variation in RMS. The effect of the third milking has been studied in the past by many researchers (Pearson et al., 1979; Gisi et al., 1986; Klei et al., 1997). The third milking seems to reduce fat percentage (Gisi et al., 1986; Klei et al., 1997), whereas a reduction to once-daily milking leads to an increase of fat content (Davis et al., 1999). However, we must remember that research on the third milking, carried out with the traditional milking parlour system, highlighted the effect of an increased milking frequency with a constant interval between milk-

Figure 2. Least square means of the percentage of milkings per day with milking interval higher than 12 hours by season in the first two years of RMS. Bar without common letter indicates that seasons differ with \( P < 0.05 \).
ings. With RMS, we must also consider the variability of the intervals between milkings. Generally, the introduction of a RMS, increasing milking frequency, leads to a reduction in fat content ranging from 1 to 5 g kg⁻¹ of milk (Abeni et al., 2002; Billon and Tournaire, 2002; Everitt et al., 2002; Table 1), whereas Klungel et al. (2000) did not find a decrease in milk fat content after RMS introduction. Bruckmaier et al. (2001) pointed out that milk fat was higher after short than after long intervals since the previous milking. A similar result was obtained at our Institute (Fioretti et al., 2003). However, this seems to occur only with very short intervals (i.e., less than 4-6 hours), probably as consequence of an incomplete previous milking.

Our results on bulk milk showed a reduction in the milk fat percentage during spring and summer in both years (Figure 3), with a mean of 3.80 ± 0.25 % for the 2-year period. It seems reasonable to suppose that the reduction in fat content could be due to the season and photoperiod influences on milk quality (Bernabucci and Calamari, 1998; Dahl et al., 2000; Calamari et al., 2002), independently from the RMS effect on milking frequency.

Table 1. Effects on milk quality from different experiences after the introduction of a robotic milking system, when compared with a previous traditional system based on a twice-daily milking routine (↓ indicates a decrease, = indicates the absence of significant variations, ↑ indicates an increase).

| Reference            | Fat, % | Protein, % | Titratable acidity °SH/50 ml | Clotting time, min | Curd firmness, mm |
|----------------------|--------|------------|-----------------------------|-------------------|-------------------|
| Abeni et al., 2002   | ↓      | ↓          | =                           | =                 | ↑                 |
| Billon and Tournaire, 2002 | ↓      | ↓          |                            |                   |                   |
| Everitt et al., 2002 | ↓      | ↓          |                            |                   |                   |
| Klungel et al., 2000 | =      | ↓          |                            |                   |                   |

Figure 3. Least square means of fat percentage by season in the first two years of RMS. Bar without common letter indicates that seasons differ with \( P < 0.05 \).
**Creaming properties.**

Natural creaming of fat is a traditional technique that is currently a subject of new interest (Ma and Barbano, 2000). This process has its theoretical basis in Stoke's law (Alais, 1984): large fat globules, due to their large diameters, rise faster than small globules, and the rising speed is proportional to the square of particle diameter. However, the process is faster than the theoretical estimate. This difference is probably due to the role that immunoglobulins play in fat globule clustering (Alais, 1984).

The result of this process is fundamental in the evaluation of milk aptitude for Grana-type cheese production. In this way it is possible to achieve two main results:

a. to remove a high percentage of bacteria from the milk;

b. to standardize fat:casein ratio.

The creaming properties significantly affect the contamination level of the milk that reaches the kier. This is fundamental for reducing the contamination of clostridia spores, the main enemy of Grana ageing because they are the origin of late swelling of the whole cheese, leading to dramatic losses during cheese ripening.

The standardization of the fat:casein ratio obtained with natural creaming is very important. The fat that remains in milk after natural creaming is characterized by a small globule size, with a consequently high ratio between surface membranes and globule volume. This increased ratio may affect milk fat-derived flavour development in ripened cheeses, because it is in the membranes that the lipolytic enzymes are present. This may affect the aroma of the cheese, which can derive from the fat component. The replacement of gravity separation with centrifugation and heat treatment is not possible because it can influence the coagulation quality of milk (Bottazzi, 1976). In fact, the creaming takes place in vats during a stage when the milk is left to rest. During this rest there is a slight acidification of the milk that allows a partial demineralisation of casein micelles; this leads to improved curd features (Resmini, 1978). The two main brands of typical Italian cheese from cow milk, Grana Padano and Parmigiano Reggiano, have a different processing routine for the creaming stage. Grana Padano processes milk after 11-12 hours (if milk reaches the plant at 8-12 °C) or after 6-8 hours (if milk reaches the plant at 18-20 °C) of rest in a creaming vat.
twice daily, corresponding with the traditional milking system; the different resting time is related to the acidification requirement. Parmigiano Reggiano processes milk once daily, mixing the product of a first milking, obtained after 14-16 hours of natural creaming, with the product of a second milking, obtained after 2-3 hours of natural creaming (Resmini, 1978).

There are different laboratory methods to assess creaming properties. The first is the one that was also employed in our first period of study on bulk milk from RMS. In this method, the volume of the formed cream layer was used as an index of the creaming ability of the milk. Cream volume measurements were made after 2, 4, 6, and 24 hours, and the volumes were divided by the percentage of fat in the milk in order to express the creaming properties in cream volume percentage per 1% of fat present in the milk (Bottazzi et al., 1968).

There are no data available from literature on the creaming capacity of milk obtained with RMS. Our results regarding creaming capacity are reported in Figures 4 and 5. Creaming capacity evaluated after 6 h could be considered a good index of creaming capacity for Grana type products. The highest values were recorded in autumn and the lowest in spring (Figure 4), with a mean of 2.21 ± 0.22 % of cream volume per 1% of fat for the 2-year period. This pattern agrees with the results of Bottazzi et al. (1975) obtained with the conventional milking parlour.

The ratio between creaming ability after 6 h and that after 24 h (Figure 5) could be taken as a measure of the speed of the process compared with its endpoint. There was a mean of 80.13 ± 4.46 % for the 2-year period. During spring there was slow creaming, as can be appreciated from the ratio considered. There was an improvement in creaming speed in the second year of our work (P < 0.05). From these data, it is difficult to determine whether the differences between years and seasons were due to globule size or to other factors that may affect the creaming process, such as immunoglobulins and bacteria that interact in the clustering phase.

To evaluate whether differences in creaming features between years, seasons, or milking-related factors are due to differences in fat globule size distribution, research on fat globule dimensions from individual milkings is in progress.

Figure 5. Least square means of the ratio between creaming activity after 6 h and creaming activity after 24 h, as a percentage of the latter, by year and season in the first two years of RMS. Bar without common letter indicates that seasons differ with P < 0.05. The difference between years of sampling was significant (P < 0.05).
Milk protein and its technological features

Nitrogen fractions.

True proteins are constituted of about 80% caseins and 20% whey or milk serum proteins (Dalgleish, 1992).

The caseins are phosphoproteins that precipitate at pH 4.6, whereas whey proteins remain soluble (Dalgleish, 1992).

All caseins have distinct polypeptide chains and contain different numbers of phosphorilated serine residues. Caseins can be divided into Ca-sensitive (αs- and β-caseins) and Ca-insensitive (κ-casein). αs-casein forms small aggregates, mainly tetramers, in solution at neutral pH, and β-casein forms larger aggregates, containing about 30 molecules. The micelles have an outer surface enriched with κ-casein and an interior enriched with β-casein, with α-caseins being present both on the surface and in the interior. The properties of the casein micelles are largely governed by the properties of the “coat” of κ-casein. A proportion of the β-casein can be attacked by the proteolytic enzyme plasmin, which is derived from blood, obtaining the production of proteose peptones and γ-caseins (Dalgleish, 1992).

The renneting features of caseins may be altered by the action of endogenous proteases that can be present in milk. The most important endogenous protease is the plasmin, which derives from the activation of its inactive form, the plasminogen, from the action of plasminogen activators (PA). The effects of the stage of lactation on the activity of this protease system have not been fully clarified. In fact, Politis et al. (1989a) reported that, at the beginning of lactation, the plasminogen to plasmin ratio is about 6.55 and it is reduced to 3.29 at the end of lactation; in contrast and more recently, Nicholas et al. (2002) reported an increase of that ratio in mid and late lactation. However, Gilmore et al. (1995) pointed out that PA activity in the casein fraction during late lactation is ~ 2-fold higher than during early or mid lactation. Plasmin activity was also associated with milk pH. Correlation coefficients between plasmin and α-casein, β-casein, and pH were −0.14, −0.27, and 0.19 (Politis et al., 1989b). If we consider that the optimum pH for PA activity is 7.8 (Schaar, 1985), and the evidence of an increased milk pH from 1× in late lactation obtained by Lacy-Hulbert et al. (1999), we can sup-
pose that this higher pH could determine increased PA activity in late lactation. The relationship between plasmin activity and milking frequency could be derived from the works of Stelwagen et al. (1994) and Sorensen et al. (2001): changing from 1× to 2× (Stelwagen et al., 1994) and from 2× to 3× (Sorensen et al., 2001) there was the effect of increased milking frequency reducing the time available for plasminogen to be converted to plasmin. These results suggest that RMS could improve milk casein content through an increased milking frequency. In addition, in udder-halves milked once daily, low casein to whey protein ratios, high Na⁺ to K⁺ ratios, and high somatic cell counts were indicative of changes in epithelial permeability (Boutinaud et al., 2003), confirming the probable positive role of RMS on cheese-making properties through the possible increase in milking frequency.

**Titratable acidity and pH.**

The titratable acidity of milk is a measure of the binding ability of caseins. The principal binding sites for Ca²⁺ are the phosphoseryl residues. The phosphate moieties of the phosphoseryl groups are titrated between pH 6 and 7.

Titratable acidity plays a fundamental role in all phases of milk rennet-coagulation:

- reactivity between rennet and casein
- aggregation rate of para-casein micelles
- syneresis ability of the curd.

The pH, strictly correlated with the titratable acidity, markedly affects the rate of hydrolysis of the κ-casein by the chymosin.

The increase from two to three milkings seems to improve milk titratable acidity (Pallavicini et al., 1981).

Decreased milking frequency of cows in late lactation increased milk pH (Kelly et al., 1998).

From our first results (Abeni et al., 2002) protein content slightly decreases after the introduction of an RMS (1.8 g kg⁻¹ of milk), as obtained also by Everitt et al. (2002) and Klungel et al. (2000), whereas Billion and Tournière (2002) did not find variation in protein content after RMS introduction (Table 1). The protein content in milk from our RMS had a mean of 3.53 ± 0.10 % throughout the 2-year period; it was significantly lower in the second year, causing an increase in the fat:protein ratio, and decreased in spring and summer (P < 0.05; Figure 6). However, the titratable acidity was not affected and remained at a level (Figure 7) as good as it had been before the introduction of robotic milking (Abeni et al., 2002). Throughout the 2-year period, titratable acidity had a mean of 3.43 ± 0.09 °SH/50 ml, and milk pH had a mean of
6.72 ± 0.02 units. From this outcome, it seems reasonable to suppose that the milk protein decrease had not significantly affected the casein content. The ratio between titratable acidity and protein also supports this line of argument. This ratio, expressed as (°SH/50 ml):(protein %), was higher ($P < 0.05$) in spring and summer (1.000 and 0.996, respectively) than in autumn and winter (0.938 and 0.952, respectively), confirming the observation of Calamari et al. (2002) on the effect of season and photoperiod on milk protein features. These results were not affected by the somatic cell count, considering the absence of significant variation between seasons in this latter variable.

**Clotting features.**

Similarly to titratable acidity, clotting features can also be affected both by the storage technology associated with RMS and the different milking frequency. Raynal and Remeuf (2000) pointed out that clotting ability of bovine milk is more sensitive to cold storage damage than caprine and ovine milk. In particular, they recorded a 30% increase of the clotting time, compared with the initial value, after 48 hours of cold storage (Raynal and Remeuf, 2000).

On the other hand, the increased rest in the udder seems related to an increased activation of plasminogen to plasmin, the dominant proteases in native milk. Data obtained by Stelwagen et al. (1994) from a comparison between once and twice-daily milking showed that in the first case there was a higher plasmin activity; this protease in particular leads to the degradation of α- and β-caseins. This result was confirmed by the observation of Sorensen et al. (2001), who emphasised that thrice-daily milking allows a higher casein number compared to twice-daily milking, according to the reduced extent of α- and β-casein degradation.

O’Brien et al. (2002) obtained no effects of milking frequency on rennet coagulation time (RCT) and curd firmness at 60 min (A60) with once-daily milking and twice-daily milking.

In addition, account should be taken of the results obtained by Bastian et al. (1991), who reported that plasmin activity did not influence milk-clotting parameters; clotting time decreased as pH decreased and as protein and fat increased; increased protein and fat improved the firming rate of curd (Bastian et al., 1991).

Our results on renneting time showed a mean of 22.74 ± 1.80 min, with an increase in summer values (Figure 8). This pattern was also observed with the milking parlour system, as an effect of...
warm climate on dairy cows (Calamari and Mariani, 1998) and was not fully eliminated in autumn. This result therefore did not seem to be related only to RMS introduction. The worsening from cold to warm seasons in the curd firming rate was evident (Figure 9) and agrees with data obtained in the milking parlour (Calamari and Mariani, 1998), with a mean of 6.03 ± 1.48 min for the 2-year period. Curd firmness had a mean of 31.78 ± 3.52 mm throughout the 2-year period and its seasonal pattern is reported in Figure 10. The worsening in clotting features during warm months is also well documented with the milking parlour (Calamari and Mariani, 1998). The reduction in milking frequency, more pronounced in the summer of our second year, does not seem to be the cause of this result, considering the similar trend of clotting features during both years. However, the effect of an extended milking interval during summer on clotting features must be further studied.

Specific comparative trials are now in progress at our Institute to evaluate the effect of the different milking system on clotting features not only at a laboratory scale, but also on a dairy plant scale. In fact, a trial with fifteen separate cheese-making processes with the Grana technology started in the winter of 2003; the effects on cheese quality will be evaluated at two intervals: 9 and 12 months.

Conclusions

Looking at the indications in the literature and at our first results on bulk milk, it seems evident that the RMS modifies some qualitative parameters when compared to the traditional milking parlour system. If a reduction in milk fat and protein content takes place, creaming activity, titratable acidity and clotting features from laboratory tests seem to remain at a level acceptable to the dairy industry. However, the results of specific studies on individual milk and on cheese-making properties on a plant scale will provide the best indications.

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Figure 9. Least square means of curd firmness (mm) by season in the first two years of RMS. Bar without common letter indicates that seasons differ with $P < 0.05$. 

![Figure 9](image-url)
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