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# Evaluation of Milk Enzymes and Electrolytes, Plasma Metabolites, and Oxidative Status in Twin Cows Milked in an Automatic Milking System or Twice Daily in a Conventional Milking Parlor

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## ABSTRACT

The aim of this paper was to evaluate the effects of automatic milking (AM) on milk enzymes and minerals related to mammary epithelial integrity in comparison with twice-daily conventional milking (CM). One cow from each of 6 pairs of twins was assigned to be milked with AM or with CM throughout first lactation. Milk production was recorded and milk samples were collected at 4, 11, 18, 25, 32, and 39 wk of lactation (WOL) to determine fat and protein content, somatic cell count, pH, plasminogen (pl) and plasmin (Pl) activities, Na, K, and Cl. Body condition score was monitored; blood samples were collected to determine energy-related metabolites in the first third of lactation (14 WOL), and plasma oxidative status throughout lactation. Overall mean and standard deviation of milking frequency (MF) in AM were 2.69 and 0.88, respectively. Milk production, fat and protein contents, and somatic cell count did not differ between milking systems. The pl and pl+Pl activities were lesser in AM than in CM. Milk pH was greater in AM than in CM. Milk Na, K, Na/K ratio, and Cl did not differ across the whole lactation. Milk pH had a positive correlation with milk Pl activity ($r = 0.41$), Na ($r = 0.37$), and Cl ($r = 0.40$) concentration, and negative correlation with the $\log_{10}$ of pl/Pl ratio ($r = -0.47$). The milk Na/K ratio had a positive correlation ($r = 0.55$) with milk Pl activity. Milking system (MS) did not seem to affect mammary epithelial permeability. The differences in enzymatic (proteolytic) activity due to the MS, probably related to daily MF, lead one to suppose that the quality of the protein fraction for the cheese-making process was preserved better with AM than with CM, even if differences in pH might negatively interfere. No difference was detected in BCS, and in plasma concentration of triglycerides and nonesterified fatty acids, whereas plasma cholesterol concentration during the first 10 WOL was lesser in AM than CM. Oxidative status, measured by plasma reactive oxygen metabolites and thiol groups, did not differ between MS throughout the whole lactation. These results suggest that early lactation of AM primiparous cows may give rise to crucial situations: for milk production, when a low MF may impair further mammary cell proliferation; for milk quality, if an irregular MF, with prolonged milking intervals, leads to an increased milk pH with increased conversion of pl to Pl.

**Key words:** automatic milking, plasmin, plasminogen, milk electrolyte

## INTRODUCTION

The automatic milking (**AM**) system represents an opportunity to achieve 2 goals: to relieve the farmer from the labor-intensive routine of the conventional milking (**CM**) parlor; to allow a voluntary increase in milking frequency (**MF**) of the cow, which has been associated with an increase of 2 to 8% of milk production for multiparous cows (Svennersten-Sjaunja and Pettersson, 2008). However, there are some cases in which the introduction of AM caused a reduction of MF and milk production (see review of Pirlo et al., 2005).

Automatic milking implies variability in MF, generally affected by several factors related to the cow (parity, DIM, health status) and management (Spolders et al., 2004). As a cascade, different MF within AM, also characterized by irregular milking intervals, may affect milk production (Abeni et al., 2005a; Bach and Busto, 2005; Speroni et al., 2006), milk quality (Abeni et al., 2005b), and some metabolic aspects (Abeni et al., 2005a).

There is abundant evidence that the rate of milk secretion is directly correlated with MF, as a result of the mechanisms related to the local control of milk

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secretion (Knight et al., 1998). Long intervals between milkings have been reported to decrease mammary blood flow (Delamaire and Guinard-Flament, 2006a) and downregulate the udder's ability to extract nutrients from the blood (Delamaire and Guinard-Flament, 2006b).

In addition, MF acts through the effect of milking interval on the time available for plasminogen (**pl**) to be converted to plasmin (**Pl**), resulting in an increased pl to Pl ratio changing from twice-daily (2 ×) to thrice-daily (3 ×) milking (Sorensen et al., 2001). In healthy udders, Pl is the main endogenous protease, which, in turn, is formed in milk after specific cleavage of pl, its inactive zymogen derived from blood. The concentrations of both pl and Pl in milk increase as lactation progresses, due to increased activation of pl to Pl and also to greater leakage of pl from serum into milk (Stelwagen et al., 1994). The assessment of the effects of AM on pl and Pl activities is of particular concern for milk that must be transformed into typical Italian long ripening cheeses because they can reduce efficiency of transformation and product quality (Pirlo et al., 2004).

A common way of assessing paracellular permeability in the lactating mammary gland is to measure the solute content of milk; the concentration of lactose and K in milk decreases (moving down their concentration gradients), whereas those of Na and Cl increase when the junctions become leaky (Stelwagen et al., 1999; Shennan and Peaker, 2000; Boutinaud et al., 2003; Shamay et al., 2003; Delamaire and Guinard-Flament, 2006b). With a study in which different MF (2 × vs. 3 ×) on half-udder of the same cows were adopted, Sorensen et al. (2001) concluded that mammary epithelial integrity (assessed by milk Na to K ratio) was greater in the half-udder that was milked 3 ×.

Wiktorsson et al. (2003) investigated some physiologic and metabolic aspects of the cow in an AM system throughout the first 19 wk of lactation (**WOL**). They showed that primiparous cows may experience a severely negative energy balance in early lactation, with a greater body tissue mobilization than that of the multiparous cows, when a high MF occurs in the AM. Wenzel and Nitzschke (2004) compared CM and AM to study the effects on the incidence of ketosis, and they did not find any significant differences in serum glucose, BHBA, and urea. Abeni et al. (2005a) investigated plasma metabolites in primiparous cows in AM system compared with those in CM system and did not find any significant differences in milk production, BCS, and energy-related metabolites (glucose, NEFA, BHBA, and triglycerides) during the first 22 WOL.

Metabolism and environmental factors can cause oxidative stress as result of an imbalance between endogenous production of reactive oxygen metabolites (**ROM**) and neutralizing capacity of antioxidant mechanisms. Plasma level of ROM is considered an indicator of free radical production (Bernabucci et al., 2005). The markers of oxidative status were chosen because they have all been implicated in the pathways that link oxidation to pathologic processes that may affect milk production, reproduction, and immunity (Bernabucci et al., 2005). Total thiol groups of plasma (**SHp**) represent the sulfhydryl groups of albumin, L-cysteine, and homocysteine; they are considered a significant element of the extracellular antioxidant defense system against oxidative stress and can be used to describe antioxidant potential in dairy cows (Bernabucci et al., 2005).

The hypothesis of increased variability in milk production due to the AM system, together with insufficient information on cow metabolic status from field trials adopting a forced traffic system, justifies monitoring energy-related metabolic aspects when CM and AM are compared.

The objective of this study was to compare the effects of the variation of MF related to the adoption of an AM system on cow mammary epithelial integrity and milk proteolytic enzymes during the first lactation. In addition, metabolic profile and oxidative status of the cows were monitored to assess possible interferences on the results of milk production and milk quality aspects.

## MATERIALS AND METHODS

### *Animals and Husbandry*

One cow from each of 6 pairs of twin Italian Friesian heifers was assigned to be milked with AM or with CM throughout first lactation. Two months before calving, heifers were scored for BCS, according to Edmonson et al. (1989). All of the heifers calved from March 15 to August 20, 2003; the longest interval between calvings within a twin pair was 12 d. At the beginning of lactation, the animals were introduced into 2 similar herds, each composed of a total of 45 cows, which were similar for average milk production and parity.

Both herds were housed in the same free stall barn with cubicles. On one side of the barn there was an 8+8 herring-bone milking parlor; on the other side there was a single box AM system (DeLaval VMS, DeLaval, Tumba, Sweden). Both groups were fed with the same TMR distributed once daily (at 0800 h). Cows in AM system also received a concentrate supply in the milking stall: 1 kg/d every 10 kg of milk production, on average. This concentrate supplied in the milking stall had a chemical composition very similar to the TMR chemical characteristics (Table 1). Cows in CM received an addition of concentrate supplied with TMR

**Table 1.** Average composition, chemical analysis, and nutritive value (% of DM) of TMR fed to twins milked in a conventional milking parlor (CM) or in a single box automatic milking system (AM)

| Item | CM | AM[1] |
|---|---|---|
| Ingredient | | |
| Fescue hay | 8.6 | 9.6 |
| Alfalfa hay | 7.8 | 7.2 |
| Corn silage | 33.2 | 34.0 |
| Cottonseed whole | 8.1 | 8.1 |
| Corn grain (70% ground and 30% steam flaked) | 22.2 | 21.1 |
| Commercial protein concentrate[2] | 19.2 | 19.1 |
| Vitamin and mineral premix[3] | 0.9 | 0.9 |
| Nutrient | | |
| CP | 15.1 | 14.9 |
| Ether extract | 4.78 | 4.77 |
| NDF | 32.77 | 33.23 |
| ADF | 20.26 | 20.71 |
| Starch | 27.61 | 27.05 |
| $NE_L$, Mcal/kg of DM | 1.57 | 1.57 |

[1]Cows in AM also received a concentrate supply in the milking stall (1 kg/d every 10 kg of milk production, on average) and 1 kg of DM contained 177 g of CP, 37 g of ether extract, 292 g of NDF, 120 g of ADF, and 245 g of starch.

[2]1 kg contained 12 g of Ca, 7.8 g of P, 23 mg of Fe, 6 mg of Cu, 110 mg of Mn, 0.3 mg of Co, 165 mg of Zn, 0.9 mg of I, 0.02 mg of Se, 45,000 IU of vitamin A, 2,000 IU of vitamin $D_3$, and 65 mg of vitamin E.

[3]1 kg contained 145 g of Ca, 70 g of P, 1,350 mg of Fe, 400 mg of Cu, 2,500 mg of Mn, 50 mg of Co, 500 mg of Zn, 115 mg of I, 20 mg of Se, 1,000,000 IU of vitamin A, 150,000 IU of vitamin $D_3$, 5,000 mg of vitamin E.

that, ranging between 0.5 to 1.5 kg/d at the planned DMI level for the primiparous animals of the 2 groups, achieved a balance in average energy and protein intake in the 2 herds (Table 1). Routine milking times in the parlor started at 0530 h and at 1630 h. Ingredients of the TMR in both groups are reported in Table 1.

In the AM system area, selectively forced cow traffic (as defined by Harms et al., 2002) was applied. The feeding area was separated from the resting area by 1-way gates, which allowed the cow free access to the cubicles without being milked. However, they were obliged to pass the AM area before entering the feeding area, with a bypass consisting of a preselection gate: cows that had recently been milked (less than 5 h, with the exception of passages after an incomplete milking) did not have to pass the milking stall and the waiting area in front of it and were deviated directly to the feeding area, whereas the others were admitted to the milking stall. The layout of our barn with AM was reported in Abeni et al. (2005a). The AM system was always accessible except during cleaning at 0400, 1200, and 2000 h for about 30 min each time. Twice a day (0530 and 1730 h), any cow that had not been milked during the last 12 h was fetched to the AM unit to avoid milking intervals greater than 12 h. Health disorders of each heifer were recorded and classified during the trial.

Milk production was recorded continuously with AM, using a daily mean calculated on 4 consecutive days as representative of a week for statistical analysis, whereas milk production of cows with CM was recorded weekly (for 2 consecutive milkings). Data on traffic of each twin cow with AM were obtained from the recorded passages through the preselection gate and in the milking unit.

### *Sampling*

Blood samples were obtained before the morning distribution of TMR, at 0730 h. The bleeding moment, with respect to calving date, was −14, 1, 7, 14, 28, 42, 70, 98, 154, 210, and 266 d. With the same schedule, BCS was evaluated on each heifer. A single blood sample was collected from the jugular vein via venipuncture, using 10-mL Li-heparin Venoject tubes (Terumo Europe, Leuven, Belgium). Blood samples were immediately placed in an ice bath, where they were stored until they were processed, within approximately 30 min from withdrawal. Packed cell volume was determined using a hematocrit centrifuge; then, plasma was separated by centrifugation (2,850 × *g* for 20 min), and 4 subsamples were stored immediately at –20°C until analyzed.

Milk production in CM system was recorded weekly for 2 consecutive milkings throughout the trial; milk production in AM system was automatically recorded by the system, and the mean of 4 consecutive days was calculated as representative of each WOL; milk samples were collected at 4, 11, 18, 25, 32, and 39 WOL.

### *Laboratory Analyses*

Feedstuffs were analyzed for dry matter, CP, crude fiber, NDF, ether extract, ash, and starch content (Martillotti et al., 1987). The values of $NE_L$ were estimated according to NRC (2001).

The blood metabolites were analyzed at 37°C by a clinical analyzer (ILAB 650 photometer, Instrumentation Laboratory, Lexington, MA). The plasma glucose, urea, cholesterol, and triglyceride concentrations were determined using commercial kits (IL Test Kit reagent, Instrumentation Laboratory). In detail, the plasma levels of glucose and cholesterol were determined by a bichromatic analysis; the plasma levels of urea and triglycerides were determined by a kinetic fixed time analysis. The plasma levels of NEFA were determined by an enzymatic-colorimetric test (C-Test Wako, Wako Chemicals GmbH, Neuss, Germany). Plasma levels of SHp and ROM were run with colorimetric methods (Diacron, Grosseto, Italy).

**Table 2.** Results from ANOVA of milk production, BCS, and blood metabolites according to milking system (CM = conventional milking parlor; AM = automatic milking system), and significance of the factors with the selected model

| | CM | | AM | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Mean | SE or CI[1] | Mean | SE or CI[1] | MS[2] | WOL[3] | MS × WOL |
| Milk production, kg/d | 23.52 | 0.58 | 23.69 | 0.51 | NS | NS | NS |
| BCS, score | 2.92 | 0.04 | 2.91 | 0.04 | NS | 0.007 | NS |
| Packed cell volume (PCV), L/L | 0.314 | 0.005 | 0.312 | 0.005 | NS | 0.136 | NS |
| Plasma glucose, mmol/L | 3.861 | 0.085 | 3.625 | 0.083 | 0.073 | NS | NS |
| Plasma urea, mmol/L | 3.746 | 0.159 | 3.858 | 0.155 | NS | 0.001 | 0.102 |
| Plasma total cholesterol, mmol/L | 3.922 | 0.184 | 3.252 | 0.179 | 0.014 | <0.0001 | NS |
| Plasma triglycerides, mmol/L | 0.108 | 0.006 | 0.114 | 0.006 | NS | NS | 0.034 |
| Plasma NEFA, mmol/L | 0.366 | 0.291–0.461 | 0.387 | 0.309–0.484 | NS | 0.013 | NS |
| Plasma NEFA/total cholesterol, ratio | 0.133 | 0.018 | 0.185 | 0.018 | 0.048 | <0.0001 | NS |
| Plasma ROM,[4] mg of $H_2O_2$/100 mL | 12.45 | 0.30 | 12.52 | 0.30 | NS | NS | NS |
| Plasma SHp,[5] μmol/L | 213.8 | 10.2 | 207.0 | 10.2 | NS | 0.002 | NS |

[1]SE or CI = standard error was reported for normally distributed variables, whereas confidence interval (95%) was reported for those variables that were processed with ANOVA after a log-transformation.

[2]MS = milking system.

[3]WOL = week of lactation.

[4]ROM = reactive oxygen metabolites.

[5]SHp = plasma thiol groups.

Milk fat and protein content were determined with a Fourier-transform infrared analyzer (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark); SCC was determined with a flow cytometry instrument (Fossomatic 5000, Foss Electric). Milk pH was determined immediately after sampling (Crison Instruments, Alella, Spain). All milk samples were assessed for Pl and pl activities. In detail, the determination of Pl was performed in duplicate in 96-well microtiter plates containing 220 μL of 0.1 *M* Tris-HCl buffer (pH 7.4), 0.6 m*M* of D-Val-Leu-Lys-4-nitroanilide (VAL, chromogenic substrate), and 30 μL of sample. The assay for pl was carried out accordingly, but 30 plough units of urokinase were added to the reaction mixture to achieve complete activation of the proenzyme. In all cases, 3 wells in which samples were replaced by buffers that served as blanks to detect spontaneous breakdown of the substrate. Reaction mixtures were incubated at 37°C for 3 h, and absorbance at 405 nm was read at 30-min intervals with a microplate reader (DV 990 BV 4/6, Gio De Vita & Co., Rome, Italy). The rate of nitroanilide formation was measured from the linear portion of the absorbance vs. time curve. Plasminogen activity was calculated as the difference between total activity and Pl activity. Plasmin and pl activities were expressed as units, one unit being the amount of enzyme that produces a change in absorbance at 405 nm of 0.1 in 60 min. The chromogenic substrate was purchased from Sigma Chemical Co. (St. Louis, MO); urokinase was from ICN (ICN Biomedicals Inc., Aurora, OH); all other reagents were of analytical grade purity. Milk concentrations of Na, K, and Cl were determined using an indirect potentiometric method by ion-selective electrodes.

### *Statistical Analysis*

Normal distribution of blood and milk variables was tested with the Shapiro-Wilk test before further analyses; therefore, not-normally distributed variables were log-transformed to obtain a normal distribution of the values for their submission to ANOVA. The experiment was a randomized block design, with milking system (**MS**; AM vs. CM), WOL, and MS × WOL as main factors, with cow repeated in time. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used, with the prepartum control as a covariate (only for BCS and blood data). The cow nested within MS × WOL was used as the subject for the test of fixed effects. A first order autoregressive (AR1) structure of covariance was selected based on the results from fit statistics Akaike's information criteria and Bayesian information criteria. Least squares means and SE are reported in figures for the selected variables. For not-normally distributed variables, back-transformed values were presented in tables and figures, and CI instead of SE was considered as a dispersion index. If a significant *F*-test was detected ($P < 0.05$), interactions were evaluated using the PDIFF option in SAS and also highlighted in figures when $P < 0.05$; a trend in data was considered when $P < 0.10$.

The same statistical procedure was used in milk production data analysis, the only difference being a greater number of observations in time and without

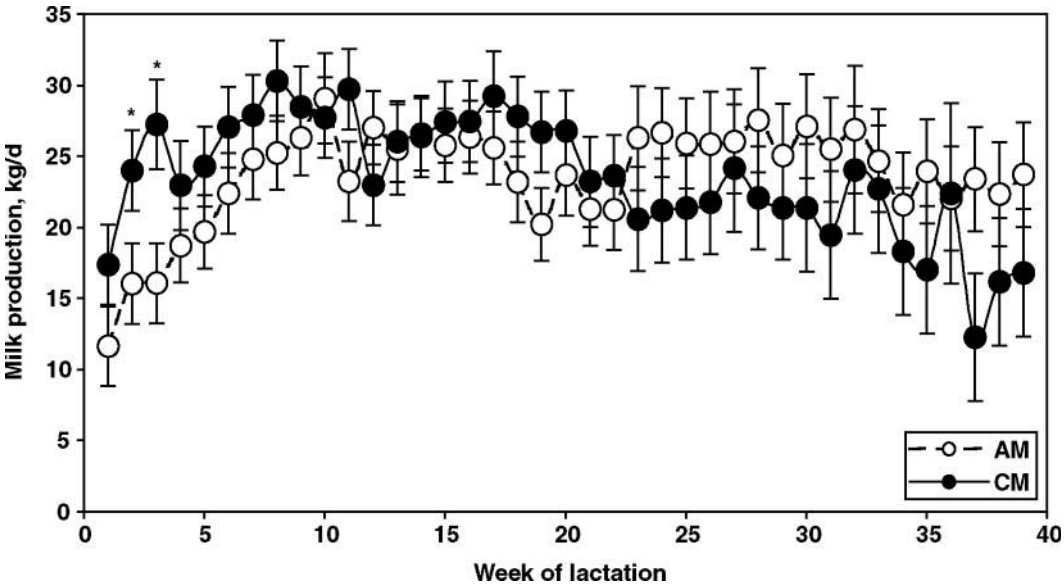


**Figure 1.** Daily milk production in the conventional milking system (CM) and in the automatic milking (AM) system throughout the experimental period of 39 wk. Data represent least squares means ± SEM. The asterisks indicate that means within week of lactation differed for $P < 0.05$.

covariate in the model. Milking frequency in the AM was analyzed by the same statistical procedure using a model considering only WOL as main effect; the same model and a model with only an intercept (Gygax et al., 2007) were performed using milking frequency minus 2 as a dependent variable to test whether the MF in the AMS differed from the constant predefined value in CM. Simple correlations among milk features were calculated and, where significant ($P < 0.05$), were reported in the text.

## RESULTS

### *Milk Production, Milking Frequency, and Plasma Metabolites*

The main statistics output on milk production, BCS, and blood features is shown in Table 2. The overall milk production was not affected by MS, but milk production at 2 and 3 WOL was lesser in AM than in CM (Figure 1).

The average milking frequency in the AM was 2.69 ± 0.6, and it was different from 2 ($P < 0.05$). However, it showed a pattern throughout lactation: the average milking frequency minus 2 for 1 to 4 WOL, 5 to 11 WOL, 6 to 18 WOL, 19 to 25 WOL, 26 to 32 WOL, and 33 to 39 WOL is shown in Figure 2. The same figure shows the absence of difference in MF between MS during the first month of lactation and, in the remaining part of lactation, a greater MF with AM than with CM.

Body reserves mobilization, as monitored by BCS, was not affected by MS and its interaction with WOL (Figure 3). There was a trend for lesser values of plasma glucose concentration in AM cows compared with CM cows ($P < 0.10$; Table 2), and this was primarily due to the lesser plasma glucose ($P < 0.05$) in AM at 2 and 4 WOL (Figure 4). Plasma urea did not differ between MS, but lesser values ($P < 0.05$) were detected in AM at 2 WOL (Figure 4). The AM cows had lesser plasma total cholesterol ($P < 0.05$; Table 2) and a greater NEFA/total cholesterol ratio ($P < 0.05$; Table 2 and Figure 5) throughout the first 14 WOL; however, this ratio was affected by the greater values in AM than in CM ($P < 0.05$; Figure 5) in the first WOL.

The oxidative status of the cows was not affected by MS (Table 2). There was a trend ($P < 0.10$) toward lesser values of ROM in AM at 2 WOL, but an opposite trend ($P < 0.10$) was observed at 22 WOL (Figure 6). Plasma SHp were affected only by WOL ($P = 0.002$; Figure 6).

### *Milk Features*

The main statistics output on milk features is shown in Table 3. Fat and protein contents, and somatic cell count did not differ between milking systems. Milk pH was greater ($P = 0.05$) in AM cows; particularly at 4 ($P = 0.037$) and at 25 ($P = 0.058$) WOL, milk pH in AM cows showed greater values than in CM cows (Figure 7). There were no effects of MS and MS × WOL on milk

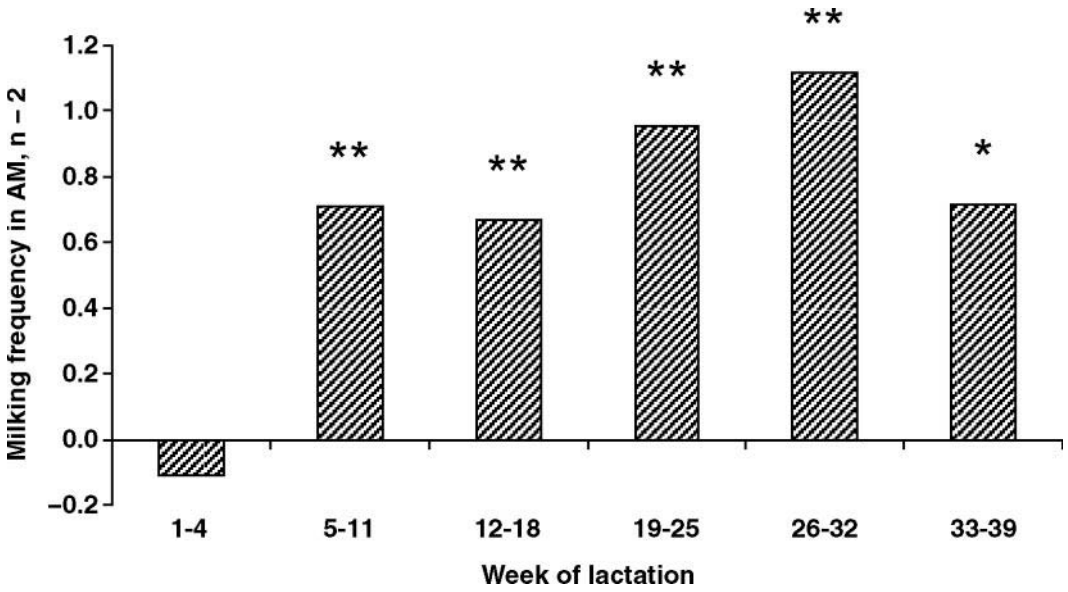


**Figure 2.** Differences between milking frequency in the automatic milking (AM) system and the conventional milking (CM) system in each period before milk sampling. Data represent least squares means (pooled SEM = 0.161). The asterisks indicate that mean within period differed from 0 for $P < 0.05$ (*) and $P < 0.01$ (**).

Na, K, and Cl contents and on milk Na/K ratio (Table 3); however, at 4 WOL, the AM milk had a trend for greater Na ($P < 0.10$; Figure 7) and Cl ($P = 0.055$; Figure 7) contents than CM milk.

Both pl (Figure 8) and total activity (pl + Pl) were lesser ($P < 0.01$; Table 3) in AM than in CM milk. The AM milk at 4 WOL had lesser ($P = 0.003$; Figure 8) pl and greater ($P = 0.010$; Figure 8) Pl, which resulted in a lesser ($P = 0.001$) pl/Pl ratio than CM milk (Figure 8).

Milk pH had a positive correlation with milk Pl (r = 0.41; $P = 0.004$), Na (r = 0.37; $P = 0.007$), and Cl (r = 0.40; $P = 0.003$) concentration and a negative correlation with the $\log_{10}$ of pl/Pl ratio (r = −0.47; $P < 0.001$). The milk Na/K ratio had a stronger positive (r = 0.55; $P < 0.0001$) correlation with milk Pl activity.

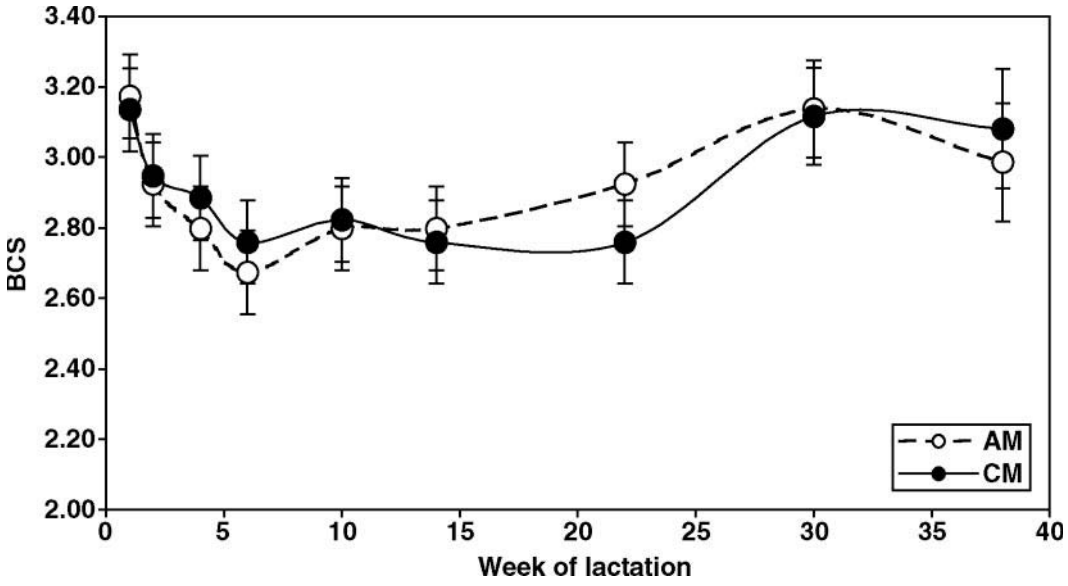


**Figure 3.** Body condition score in the conventional milking system (CM) and in the automatic milking (AM) system throughout the experimental period of 39 wk. Data represent least squares means ± SEM.

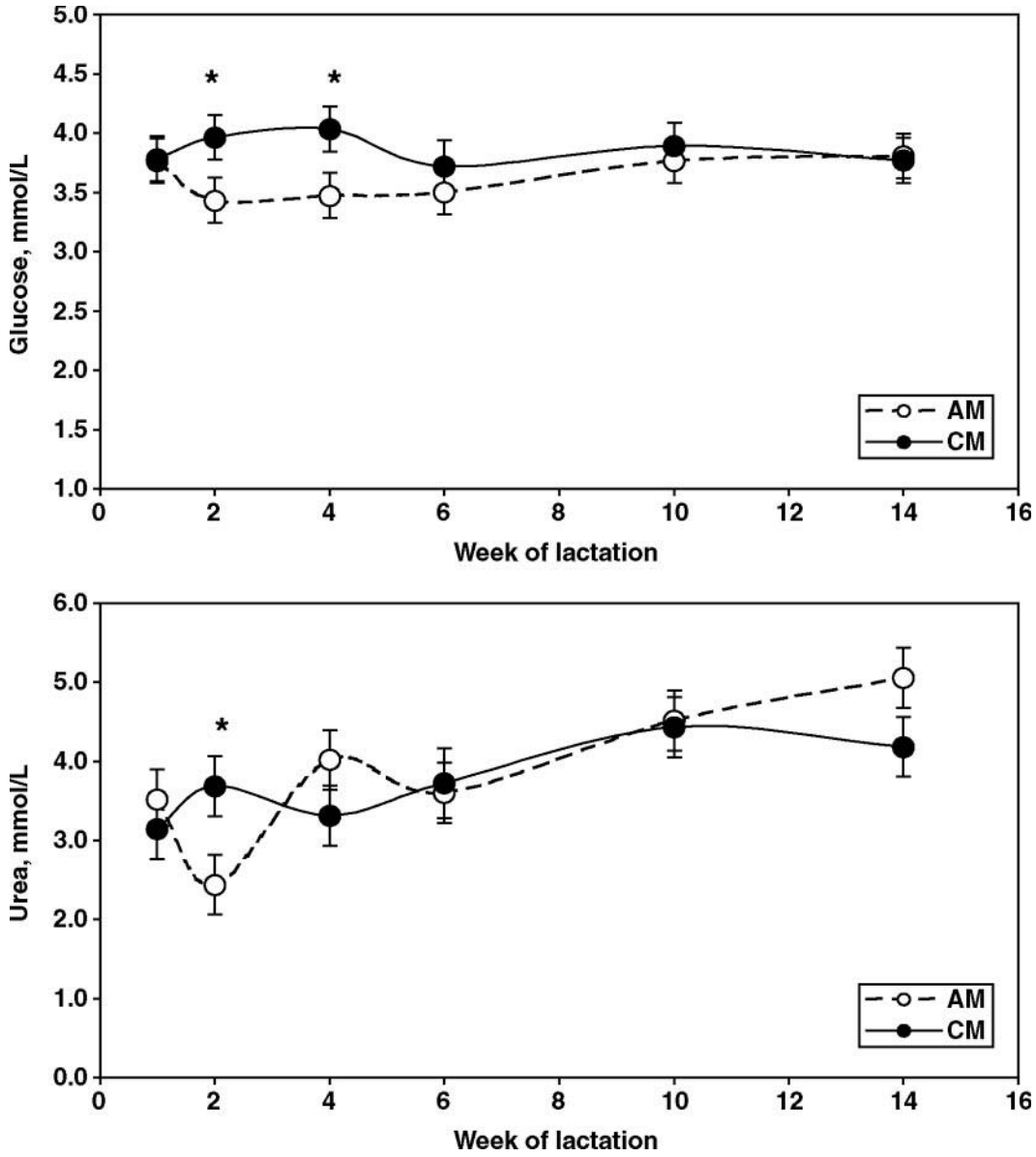


**Figure 4.** Plasma concentrations of glucose and urea in the conventional milking system (CM) and in the automatic milking (AM) system throughout the first 14 wk of lactation. Data represent least squares means ± SEM. The asterisks indicate that means within period differed for $P < 0.05$.

## DISCUSSION

### *Milk Production, Milk Composition, Plasma Metabolites, and Oxidative Status*

***Milk Production and Milking Frequency.*** In a previous study (Speroni et al., 2006), we observed increased milk production in pluriparous cows with AM, not confirmed in primiparous cows, when compared with those with CM. Even in the present study, milk production of primiparous cows was unaffected by MS, confirming our previous results (Abeni et al., 2005a; Speroni et al., 2006). Generally, increased MF with CM systems, especially during early stage of lactation, led to increased milk production (Hale et al., 2003; Dahl et al., 2004). Hale et al. (2003) reported that milk productions were 34.5, 37.8, and 37.6 kg/d from 1 to 44 WOL for 2 ×, 4 × during the first 21 DIM followed by 2 ×, and 4 × from 4 to 21 DIM followed by 2 ×, respectively. That study suggested that the increase of MF in the first 3 WOL was sufficient to elicit a carryover effect on milk production that continued into late lactation. That response seemed mainly attributable to an increased mammary cell proliferation during increased MF in early lactation (Hale et al., 2003).

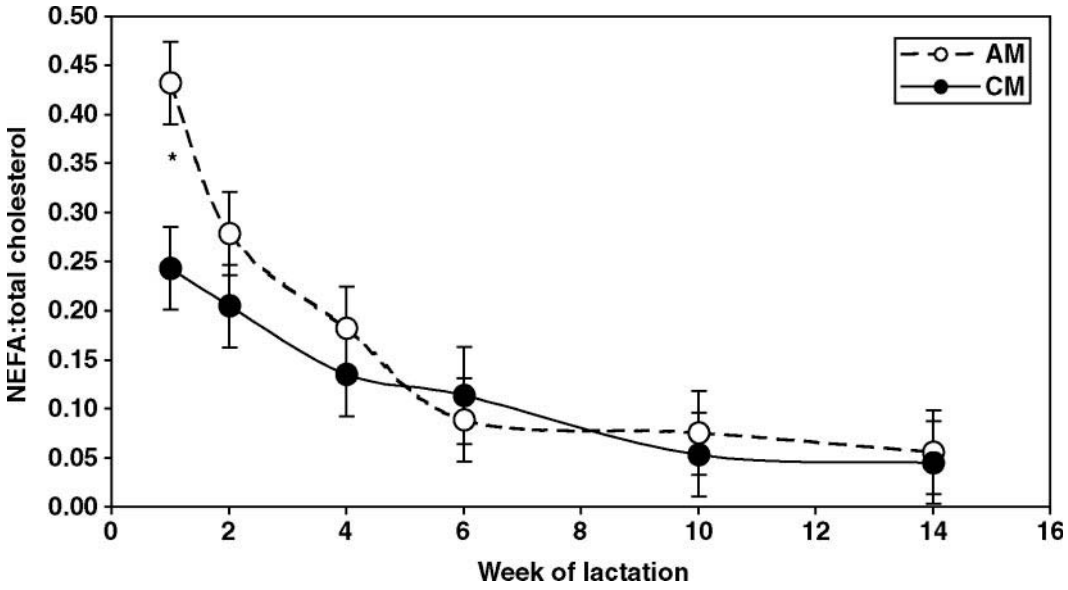


**Figure 5.** Plasma NEFA/total cholesterol ratio in the conventional milking system (CM) and in the automatic milking (AM) system throughout the first 14 wk of lactation. Data represent least squares means ± SEM. The asterisks indicate that means within period differed for $P < 0.05$.

A possible explanation for the results of our trial could be that early lactation of AM cows may have impaired the potential production by an irregular or low MF, or both, probably limiting the above-mentioned mammary cell proliferation potential (Hale et al., 2003). This issue should be taken into consideration for correct management and traffic surveillance of primiparous cows when they start to be milked in an AM system.

***Milk Composition.*** Fat and protein contents and SCC did not differ between MS, substantially confirming our previous observations on primiparous cows (Abeni et al., 2005b).

Milk SCC was found to be unaffected by MF both when once-daily (1 ×) vs. 2 × (Lacy-Hulbert et al., 1999) and when 1 × vs. 3 × (Patton et al., 2006) milking were compared. Cows milked 6 × had lesser SCC at first test day relative to those milked 3 × (Dahl et al., 2004). That difference was reflected also in reduced somatic cell scores for the first 3 mo of lactation, suggesting that MF in early lactation influences the mammary gland capacity to resist infection in addition to improving milk production efficiency (Dahl et al., 2004). In the present study, udder health appeared similar between groups, and it is possible to exclude possible interferences on mammary epithelial permeability evaluation.

***Plasma Metabolites and Oxidative Status.*** Despite a low milk production level in the first month of lactation, AM cows had lesser plasma glucose and greater plasma NEFA/cholesterol ratio than CM cows. These traits of metabolic profile suggest a slight difficulty on the part of AM primiparous cows in coping with their productive performance in early lactation. The greater NEFA/cholesterol ratio in AM than CM cows is a warning of an increased risk of developing metabolic disorders (i.e., fatty liver) when liver ability to export triglycerides does not match body fat mobilization (Drackley et al., 2005). A possible explanation for greater plasma NEFA in AM cows could be seen in the daily feeding behavior of primiparous cows within an AM herd, with longer feeding intervals for AM primiparous than CM primiparous at the sampling moment in the morning (Abeni et al., 2005a). Ketelaar-de Lauwere et al. (1998) emphasized that during forced cow traffic cows spent more time standing on the slatted floor in the feeding area, spent less time standing at the feeding gate and made fewer journeys from the lying to the feeding area. Thus, a reduced or irregular feeding activity (as arguable from Figure 2) may distress some difficulties of primiparous cows in matching their nutrient requirements, especially in early stage of lactation. The lack of differences in plasma urea and triglycerides in the present paper confirms our previous results (Abeni et al., 2005a) and agree with those of Patton et al. (2006) with different MF.

The ROM level confirmed our previous results (Calza et al., 2005) about a trend toward lesser oxidative stress in AM at an early stage of lactation, but also a trend toward greater ROM levels in AM than CM in mid to late lactation. The explanation of this result does not seem attributable, in our trial, to differences in body reserves mobilization (Bernabucci et al., 2005). The opposite pattern of ROM values in AM compared with

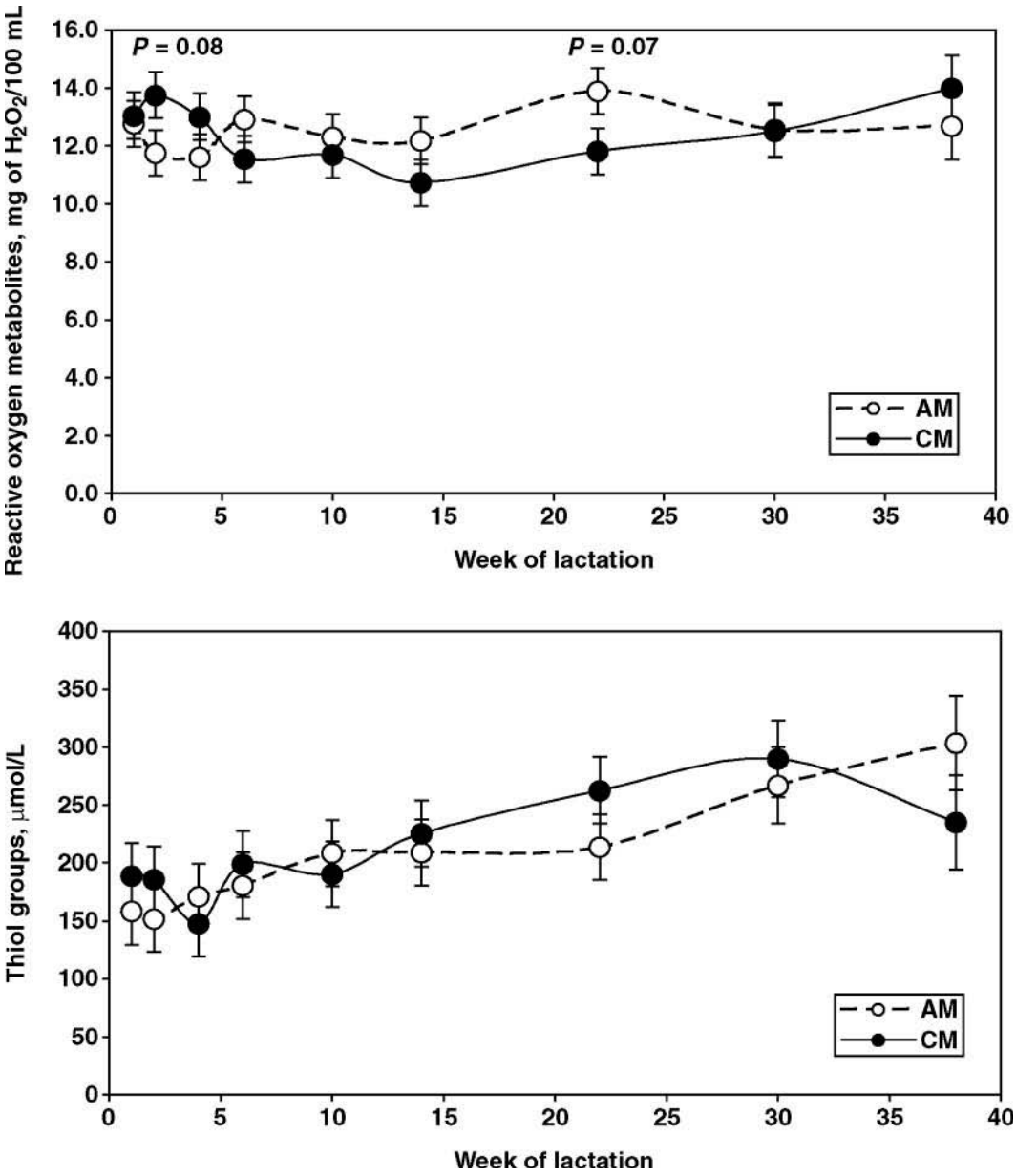


**Figure 6.** Plasma concentrations of reactive oxygen metabolites and thiols in the conventional milking system (CM) and in the automatic milking (AM) system throughout the first 14 wk of lactation. Data represent least squares means ± SEM.

that of CM suggests a relatively reduced metabolic stress in early lactation for AM, but the nature of the successive increase throughout AM lactation requires further investigation.

### *Mammary Epithelial Permeability and Proteolytic Enzymes Activity in Milk*

The present study demonstrated that milk pH was greater ($P < 0.05$) with AM, but milk Na, K, Na/K ratio, and Cl did not differ across whole lactation. A similar increase in individual milk pH in AM primiparous cows, compared with that of primiparous cows in CM, has already been observed in a previous trial at our experimental farm (Pirlo et al., 2004). From our results, the increased milk pH does not seem attributable to a different MF per se because milk pH was greater in AM even when MF was low (as observed in the first month of lactation). A possible explanation should be sought in the irregularity of milking interval within AM systems, this being the only constant feature of AM even when its MF was lesser, equal, or greater than CM

**Table 3.** Results from ANOVA of milk features according to milking system (CM = conventional milking parlor; AM = automatic milking system), and significance of the factors with the selected model

| | CM | | AM | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Mean | SE or CI[1] | Mean | SE or CI[1] | MS[2] | WOL[3] | MS × WOL |
| Fat content, % vol/vol | 3.07 | 0.16 | 3.30 | 0.15 | NS | NS | NS |
| Protein content, % vol/vol | 3.37 | 0.10 | 3.33 | 0.09 | NS | NS | NS |
| SCC, 1,000 cells/mL | 201 | 78–516 | 143 | 54–377 | NS | NS | NS |
| Milk pH | 6.674 | 0.015 | 6.715 | 0.014 | 0.05 | 0.049 | NS |
| Na, mEq/L | 22.25 | 20.10–24.64 | 22.15 | 20.26–24.21 | NS | NS | NS |
| K, mEq/L | 29.36 | 1.51 | 29.41 | 1.32 | NS | NS | NS |
| Na/K, ratio | 0.778 | 0.712–0.851 | 0.774 | 0.715–0.837 | NS | NS | NS |
| Cl, mEq/L | 17.75 | 15.65–20.13 | 18.68 | 16.73–20.85 | NS | NS | NS |
| Plasminogen, units | 31.43 | 1.63 | 23.95 | 1.49 | 0.002 | 0.001 | NS |
| Plasmin, units | 5.03 | 3.68–6.88 | 4.90 | 3.70–6.50 | NS | NS | 0.126 |
| Plasminogen/plasmin, ratio | 5.83 | 4.10–8.30 | 4.43 | 3.23–6.09 | NS | 0.019 | 0.044 |
| Total activity,[4] units | 37.23 | 1.84 | 30.40 | 1.69 | 0.009 | 0.020 | NS |

[1]SE or CI = standard error was reported for normally distributed variables, whereas confidence interval (95%) was reported for those variables that were processed with ANOVA after a log-transformation.

[2]MS = milking system.

[3]WOL = week of lactation.

[4]Total activity = plasminogen + plasmin activity.

system. Our results evidenced greater milk pH in AM at 4 WOL, together with a trend for greater milk Na and Cl contents. During this stage, primiparous cows in AM had great variability in milking interval length, in some cases greater than 12 h (Abeni et al., 2005a). Lacy-Hulbert et al. (1999) emphasized increased milk pH and Na content with 1 × compared with 2 × milking. The relationships among milk Na and Cl contents and milk pH, in the present study also lead one to hypothesize changes in ionic equilibrium not affected by udder health, which was always good in all the cows involved.

Milking system did not affect mammary epithelial permeability throughout 39 WOL, as attested by Na, K, and Cl milk content, and by Na/K ratio in milk. As reported by Sorensen et al. (2001), who compared 2 × vs. 3 ×, the Na/K ratio was inversely correlated with mammary epithelial integrity, and this ratio increased throughout the course of lactation and was always greater in 2 × (Sorensen et al., 2001). The Na concentration in milk is normally lesser than in plasma due to the presence of Na pumps only on the basolateral aspect of the secretory cell (Shennan and Peaker, 2000). An increase in the Na/K ratio is thus indicative of leaky tight junctions, allowing partial equilibration between plasma and milk (Sorensen et al., 2001).

Both pl and pl + Pl activities were lesser ($P < 0.05$) in AM. Plasmin activity was also associated with major casein components and milk pH (Politis et al., 1989). The correlation reported between milk pH and milk Pl activity (r = 0.41), in the present paper, was greater than that (r = 0.19) previously indicated by Politis et al. (1989). Plasminogen activators are present in milk and have a pH optimum of 7.8 (Politis et al., 1989). Higher pH values could increase conversion of pl to Pl with a resultant increase in proteolysis. Schaar (1985) reported a tendency for greater proteolytic activity with increasing milk pH (Politis et al., 1989). All of these considerations may be involved in the explanation of the high Pl activity and, as a consequence, the low pl/Pl ratio at 4 WOL in AM in the present study, when the greater milk pH in AM has probably led to an increased conversion of pl to Pl. In fact, one effect of increased MF is to reduce the time available for pl to be converted to Pl; Sorensen et al. (2001) observed an increased pl/Pl from 3.40 to 6.16 changing from 2 × to 3 ×. In the same study, pl concentration decreased with MF increase, suggesting that influx had been reduced, which is exactly what one would predict if uptake was passive through tight junctions that had become tighter (Sorensen et al., 2001). In our trial, the decrease in pl activity was significant in AM and was comparable with that obtained by Sorensen et al. (2001), who reported a reduction in pl activity from 42.69 to 35.43 units, changing from 2 × to 3 × (Sorensen et al., 2001).

The strong positive correlation (r = 0.55; $P < 0.0001$) in milk between Na/K ratio and Pl activity confirms the importance of the blood/milk barrier integrity in the prevention of enzymatic proteolytic activities in milk. The influx of a large number of blood components in milk has a positive effect on Pl activity, first by the increase of the pl concentration (Le Roux et al., 2003). In addition, other blood components with a main action other than pl activation do have an effect on pl conversion into Pl: epidermal growth factor, IGF-I, and prostaglandin E2, as suggested by Le Roux et al. (2003).

The differences in enzymatic (proteolytic) activity between MS, probably related to daily MF, lead to the supposition that the quality of the protein fraction for the cheese-making process was preserved better with AM than with CM, even if differences in pH might negatively interfere.

## CONCLUSIONS

Although AM is generally considered to increase milk production, this effect was not confirmed in the present study, probably because primiparous cows were compared. The metabolic profile suggests a slight difficulty on the part of AM primiparous cows in coping with their productive performances in early lactation. Considering the low MF in the first month of lactation, probably linked to low access to the feeding lane in the forced traffic system, some minor metabolic changes in AM, when compared with CM, may be related to reduced daily DMI, rather than its modified daily pattern. Early lactation of AM cows may give rise to a crucial situation because the expected increase in MF can be delayed. Nevertheless, the major milk component and udder health did not differ between MS.

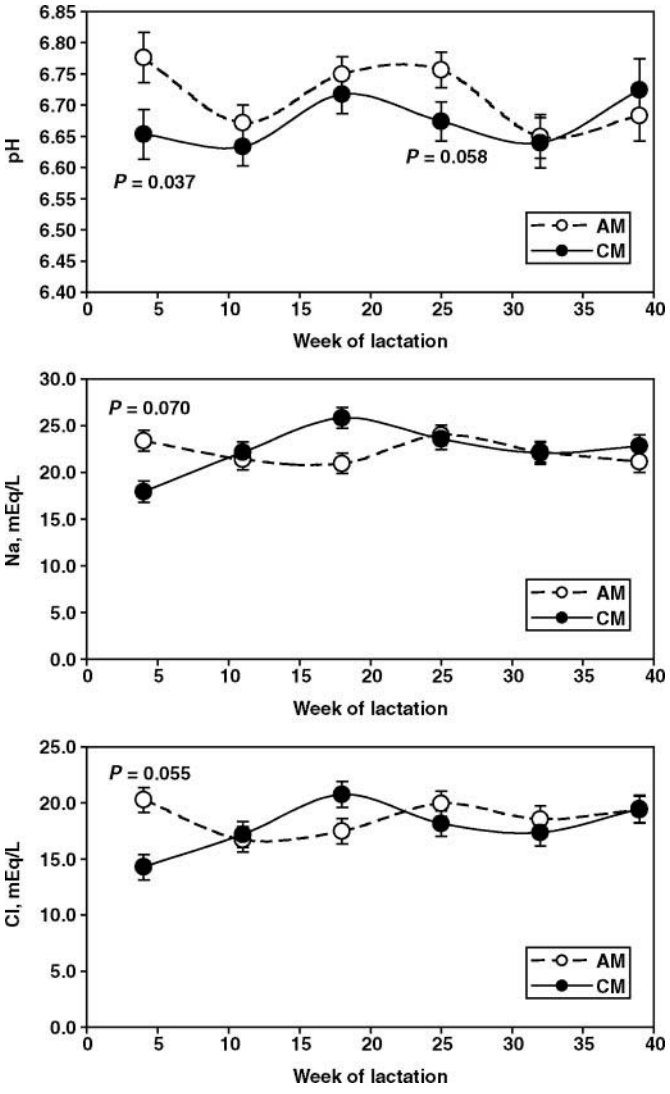


**Figure 7.** Milk pH, and Na and Cl concentrations in milk from cows in the conventional milking system (CM) and in the automatic milking (AM) system throughout the 39 wk of the trial. Data represent least squares means ± SEM. When means within week of lactation were different, or a trend was evident, the *P*-value was reported above them.

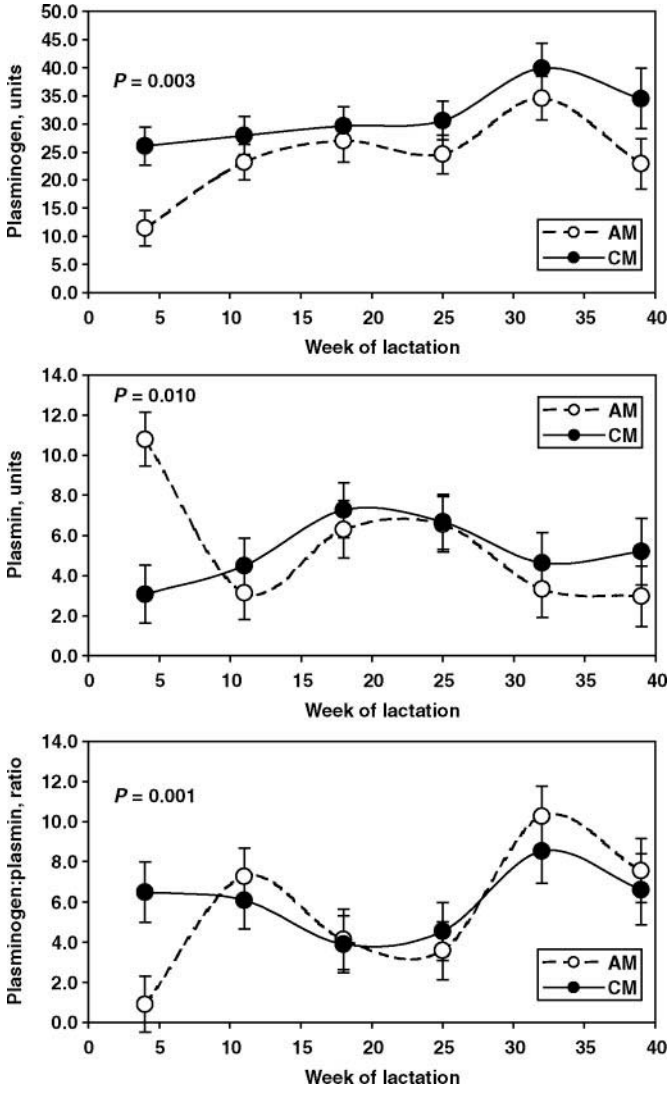


**Figure 8.** Plasminogen, plasmin, and plasminogen/plasmin ratio in milk from cows in the conventional milking system (CM) and in the automatic milking (AM) system throughout the 39 wk of the trial. Data represent least squares means ± SEM. When means within week of lactation were different, or a trend was evident, the *P*-value was reported above them.

The study showed that milk pH was greater in AM, but the lack of difference in electrolyte concentration suggests that MS did not affect mammary epithelial permeability through 39 WOL. However, the first month of lactation, when the irregular MF may lead to an excessively extended lag between milkings, was a critical point. In that stage, the greater milk pH in AM led to increased Pl activity due to the increased conversion of its precursor pl. Further studies will be necessary to assess how this increased enzymatic activity could affect milk nitrogen fractions and, as a consequence, cheese-making features.

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