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Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems

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The objective of the studies reported in this research communication was to investigate differences in composition and enzymatic activities in bulk milk samples provided from Swedish dairy farms with different management systems, i.e. automated (AMS) and conventional milking systems (CMS). A bulk milk sample was collected from each of 104 dairy farms, 51 using AMS and 53 using CMS, located in the same geographical region. Sampling took place within two consecutive days during the indoor period (October). Milk samples were analysed for contents of total fat and protein, free fatty acids (FFA), caseins and whey proteins, somatic cell count (SCC), pH, plasmin and plasminogen derived activities, and total proteolysis. Our results showed a lower protein content and higher SCC in bulk milk from AMS herds compared with milk from CMS herds. Plasmin, plasminogen and total plasmin/ plasminogen derived activities were lower in milk from AMS herds but despite this, total casein and the ß-casein fraction as % of total protein were lower in milk from AMS herds than in milk from herds using CMS. Total proteolysis was higher in milk from AMS herds, suggesting that other proteases than plasmin, e.g. cellular and bacterial proteases, contributed to the degradation of casein. This was supported by a positive correlation between SCC and total proteolysis ($P < 0{\cdot}01$), as well as a negative correlation between total proteolysis and ß-casein fraction ($P < 0{\cdot}05$). In conclusion, comparing the quality of bulk milk from commercial dairy herds using AMS and CMS, respectively, several differences were observed, suggesting a significant effect from management system.

Keywords: Plasmin, plasminogen, total proteolysis, caseins, automatic milking.

Modern dairy farmers increasingly adapt to novel management systems to reduce their workload and labour costs. In this context, automatic milking has become an established management system, reducing the workload of milking, allowing higher milking frequency without extra labour costs and enhancing milk production. However, automatic milking system (AMS) is not only about milking, consideration must be given to many aspects, including milk quality. In previous studies on the effect of AMS on milk quality, concerns related to elevated levels of free fatty acids (FFA) and increasing numbers of somatic cells (SCC) have been reported (De Koning et al. 2003). Increasing the milking frequency, as in AMS, has also been reported to correlate with a decline in plasmin and plasminogen derived activities (Svennersten-Sjaunja et al. 2007). After a decade with a rapid transition to increasingly larger dairy herds, AMS has been installed at more than 1000 dairy farms in Sweden. With almost 30% of the Swedish raw milk being provided from farms with AMS it is highly relevant to consider the influence of AMS on milk quality. The aim of this study was therefore to investigate differences in composition and enzymatic activities between bulk milk provided from commercial herds with AMS and CMS, respectively.

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Material and methods

Collection of milk samples from selected herds

Selection of 104 farms located in the same geographical region of Sweden (Mälardalen) was performed by the

dairy advisory organisation Växa Sweden with permission of the dairy companies Arla Foods and Grådö Dairy. Farms with AMS ($n = 51$) used automatic milking systems from DeLaval International AB (Tumba, Sweden) or Lely International (Maassluis, the Netherlands), and farms with CMS ($n = 53$) were equipped with traditional milking parlours and milked with regular milking intervals twice a day. Continuous calving is commonly practiced in Swedish dairy herds and the prevailing breeds in the participating herds consisted of Swedish Holstein (SLB) and Swedish Red (SRB) (Lena Loftäng, Arla Foods, personal communication). Sampling of milk took place during the indoor period. During this period, roughage generally consists of silage fed *ad libitum* while the concentrate ratio is based on individual milk yield; a routine practised on most Swedish dairy farms.

Bulk milk samples from the participating farms were collected by the central milk-testing laboratory Eurofins Steins Laboratory AB (Jönköping, Sweden). Sampling took place during two consecutive days in October in connection with regular testing for raw milk quality traits. When milk samples from the selected herds arrived to the laboratory, a sub-sample (20–30 ml) was poured into a vial and immediately frozen. The remaining milk was used for milk gross composition analysis. The collected milk samples were sent frozen and on the same occasion to the Swedish University of Agricultural Sciences (SLU), where they were subject to detailed analyses.

Milk gross composition analysis at Eurofins

Determination of the total contents of fat and protein (%) and SCC (cells/ml) was performed at **Eurofins Steins Laboratory**. Total fat and protein content was measured using Fourier Transform Infrared (FTIR; CombiFoss 6000) and SCC was analysed with flow cytometry and fluorescence (Fossomatic), both Foss Electric (Hilleröd, Denmark).

Free fatty acids

FFA concentration was determined by extraction-titration as described by Vidanarachchi et al. (**2015**). Milk fat was extracted using diethyl ether and hexane (80:20, v/v) using methyl orange as indicator. Samples were acidified using H_2SO_4 until the solution turned pink (pH 2–3), centrifuged and the supernatant was transferred to a new tube. FFA concentration was determined by titration of the supernatant with KOH in ethanol, using α-naphtholphthalein and phenolphtalein as indicator. Titration was stopped when the colour of the solution turned lilac/blue, persisting for a few seconds.

Plasmin and plasminogen derived activities

Determination of plasmin and plasminogen derived activity was performed according to De Vries et al. (2016). Plasmin and plasminogen were dissociated from casein micelles by incubation of milk with ε-amino-n-caproic acid (EACA) followed by ultracentrifugation. Plasmin activity was measured in the resulting milk serum using a chromogenic substrate, pyro-GLU-Phe-Lys-p-nitroanilide hydroxychloride and plasminogen derived activity was measured after activation with urokinase. Absorbance was registered every third minute during 120 min at 37 °C and activity was expressed as change in absorbance per time unit (ΔA405/Δt).

Total proteolysis

Total proteolysis was measured by a fluorescamine method modified from Wiking et al. (2002), based on the reaction of primary amino groups of trichloroacetic acid soluble peptides and free amino acids with fluorescamine. The milk samples were mixed with an equal volume of 24% TCA and kept on ice for 30 min before centrifugation at 16 000×***g*** for 20 min at 4 °C. Supernatant (20 µl) was mixed with freshly made sodium tetraborate pH 8, fluorescamine was added and the mixture was loaded in a 96 microwell plate. The fluorescence (excitation wavelength 390 nm and emission wavelength 480 nm) was measured after 23 min in a fluorescence spectrometer and the extent of proteolysis was expressed as leucine equivalence (eq. mM) based on a standard curve with five different concentrations (1, 0·75, 0·5, 0·3 and 0·05 mM) of 0·1 M L-leucine dissolved in 1 mM HCl. Each milk sample was analysed in triplicate.

Casein and whey protein composition

Protein separation was performed with 7100 capillary electrophoresis (CE) system (Agilent Technologies Co., USA) using unfused silica standard capillary as described by Johansson et al. (2013). Separations were performed D,L-dithiothreitol (DTT) was added to the sample buffer at the day of sample preparation in order to disrupt disulphide bridges of the milk proteins. Milk (300 µl) was mixed with 700 µl sample buffer, and defatted after centrifugation for 10 min at 10 000×***g***. Prepared samples were stored at −20 °C prior to analysis. Calculation of relative concentrations of the individual proteins was based on the peak area and expressed as a percentage of the total integrated area in the electropherogram.

Statistical analysis

Principal component analysis (PCA) was used to study the total variation in bulk milk from AMS and CMS herds using SIMCA 13·0 software (Umetrics, Umeå, Sweden). Statistical analysis was performed using IBM SPSS Statistics for calculation of differences in milk composition between management systems. Means of sample values from both systems were compared with an independent samples t-test. If the two-tailed *P*-value was 0·05, means were considered to be significantly different. Pearson correlations and their significance levels were calculated with the bivariate

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Table 1. Average composition and proteolytic activities of bulk milk from herds with automatic (AMS) or conventional (CMS) milking systems, standard error (SE) indicated

	Milking system (n = number of herds)				
	AMS (n = 51)		CMS (n = 53)		
Parameter investigated	Average	SE	Average	SE	P-value
Total protein (g/100 g)	3·55	0·07	3·63	0·07	0·005
Total fat (g/100 g)	4·29	0·09	4·49	0·09	0·102
Free fatty acids (mmol/100 g fat)	0·43	0·03	0·38	0·02	0·146
Somatic cell count (x10^3 cells/ml)	230	11·60	182	10·30	0·002
pH	6·71	0·04	6·70	0·04	0·040
Protein fractions as % of total protein					
Total casein	82·05	0·46	84·25	0·21	0·001
Total whey protein	13·88	0·12	11·50	0·39	0·001
α_{s1}-casein	*29·60*	0·16	29·47	0·23	0·662
α_{s2}-casein	6·40	0·15	6·47	0·17	0·737
β-casein	37·53	0·18	39·33	0·46	0·001
κ-casein	7·41	0·20	7·45	0·19	0·891
α-*lactalbumin*	2·75	0·04	2·34	0·07	0·001
β-lactoglobulin	11·12	0·11	9·15	0·36	0·001
Proteolytic activities:					
Plasmin (PL; U/ml)	3·63	0·18	4·35	0·16	0·004
Plasminogen (PG; U/ml)	88·96	1·33	94·64	1·32	0·003
Total activity of PL and PG (U/ml)	92·59	1·31	98·99	1·33	0·001
Total proteolysis (eq. mM leucine)	*26·50*	0·75	15·35	0·82	0·001

P-values indicate level of significance for differences between the milking systems (P-values <0·05 considered significant difference)
Due to non-normal distribution of values, statistical analyses for free fatty acids and somatic cell counts were based on their $\log_{10}$ values. Values in this table represent the back-transformed averages

procedure in SPSS, both on the complete sample set (data from both systems) as well as on subsets with either the AMS or CMS samples. Statistical analysis of SCC and FFA was performed using $\log_{10}$-values due to non-normal distribution of values.

Results and discussion

Principal component analysis

Studying the total variation in bulk milk from AMS and CMS herds, PCA was performed based on data summarised in Table 1. The score plot showed clustering of milk samples provided from AMS and CMS herds, respectively, with milk samples from AMS herds being more similar than milk samples from CMS herds (Supplementary file, Figure S1). The major quality traits contributing to differences between the two management systems included total fat and protein, casein and whey protein content, total proteolysis and pH whereas plasmin, plasminogen, SCC and FFA had less impact.

Milk components and free fatty acids

Total protein content was lower in bulk milk derived from AMS herds compared with bulk milk from CMS herds (P = 0·005; Table 1), whereas the difference in fat content was not significant. In a previous comparison of milk from AMS and CMS herds, management system had no effect on protein or fat content (Innocente & Biasutti, 2013). Lower protein content could possibly be related to a higher milking frequency with automatic milking. In studies of the effect of frequent milking on milk composition, Smith et al. (2002) reported that milk fat and protein percentages were significantly lower in herds milking 3 times a day than in those milking twice a day. In contrast to earlier studies reporting elevated FFA content in milk from AMS herds (De Koning et al. 2003) we observed no difference in FFA content between management systems (Table 1). Higher FFA in milk from AMS could partly be due to the increased milking frequency, but technical factors associated to AMS may also contribute (De Koning et al. 2003).

The average SCC in milk from AMS herds was 26% higher (230×10^3 cells/ml) than the SCC in milk from CMS herds (182×10^3 cells/ml) (P = 0·002; Table 1). Results from previous work on SCC in milk provided from AMS herds are not consistent. De Koning et al. (2003) reported significant increases in bulk milk SCC after the introduction of AMS in the Netherlands (20%) and in Denmark (8%), but not in Germany.

Plasmin and plasminogen derived activities and total proteolysis

Average plasmin and plasminogen derived activities were higher in milk from CMS herds than in milk from AMS

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herds, with total activity of plasmin and plasminogen on average 7% higher in milk derived from CMS ($P = 0{\cdot}001$; Table 1). Results are in line with those of Abeni et al. (2008) who reported that plasminogen derived activity and total plasmin and plasminogen derived activity were significantly higher in milk from CMS herds in comparison with milk from AMS herds. Sorensen et al. (2001) reported lower plasmin activity when milking frequency was increased, suggesting a better maintenance of the tight junction integrity, linked to a reduced influx of plasminogen and its activators, but also shorter time for plasminogen to be converted to plasmin between milkings. Despite lower plasmin and plasminogen-derived activities in milk from AMS herds, this milk showed on average 73% higher values for total proteolysis than milk from CMS herds ($P = 0{\cdot}001$; Table 1). These results were unexpected, suggesting that proteases other than plasmin, e.g. cellular and microbial proteases, contributed to casein degradation in AMS derived milk.

Casein and whey protein composition

Milk protein composition differed between the milking systems, with on average 2·7% lower total casein and 4·6% lower ß-casein content in milk from AMS herds ($P = 0{\cdot}001$; Table 1). Higher total proteolysis but lower plasmin activity in milk from AMS herds suggest that proteases other than plasmin must have contributed to the higher protein degradation in milk from AMS herds.

Observed correlations

Table 2 presents correlations between the investigated milk quality traits based on data from all 104 milk samples. Elevated SCC has been reported to correlate with an increase in different indigenous proteases, including plasmin (Kelly et al. 2006). This correlation was not observed in our study. In a study by Politis et al. (1989), correlation between plasmin activity and SCC was only observed when SCC exceeded 300 000 cells/ml. Considering that the average SCC in our study was substantially lower than the 300 000 in milk from both management systems, this might be an explanation for not observing a correlation between SCC and plasmin activity.

A positive correlation between SCC and total proteolysis ($P \leq 0{\cdot}01$), and a negative correlation between total proteolysis and β-casein fraction ($P \leq 0{\cdot}05$; Table 2) suggest that cellular proteases, e.g. cathepsin D, may have contributed to the higher protein degradation and lower ß-casein fraction observed in milk from AMS herds (Table 1). In their review, Kelly et al. (2006) concluded that proteolysis in low SCC milk is dominated by plasmin, however, as SCC increases, the relative significance of other enzymes increases. Further, microbial proteases might have contributed to the higher total proteolysis observed in milk from AMS herds. Due to routines at the milk-testing laboratory, analysis of

Table 2. Pearson correlations coefficients for the investigated milk quality traits calculated using the complete set of data, irrespective of milking system ($n = 104$)

	T. prot.	T. WP	T. fat	FFA	SCC	PL	PG	T-PL/PG	T. proteol.	α-LA	β-LG	α-S_1CN	α-S_2CN	κ-CN	β-CN	pH
T. prot.	1·00															
T. WP		1·00														
T. fat			1·00													
FFA				1·00												
SCC					1·00											
PL						1·00										
PG							1·00									
T-PL/PG								1·00								
T. proteol.									1·00							
α-LA										1·00						
β-LG											1·00					
α-S_1CN												1·00				
α-S_2CN													1·00			
κ-CN														1·00		
β-CN															1·00	
pH																1·00
T. prot.	1·00	−0·13	**0·59****	**−0·32****	**0·35****	0·13	**0·48****	**0·49****	−0·12	−0·19	−0·10	−0·11	−0·02	0·05	0·05	−0·11
T. WP		1·00	−0·17	0·10	**0·24***	−0·13	−0·08	−0·10	**0·37****	**0·61****	**0·99****	−0·13	−0·12	0·07	**−0·74****	0·05
T. fat			1·00	**−0·25***	−0·17	0·11	**0·38****	**0·39****	**−0·30****	**−0·33****	−0·12	0·05	−0·18	0·08	0·08	−0·07
FFA				1·00	0·15	−0·14	**−0·20***	**−0·22***	0·11	0·09	0·10	−0·05	0·03	−0·08	−0·05	0·12
SCC					1·00	−0·13	−0·07	−0·09	**0·35****	0·15	**0·24***	−0·06	−0·13	0·12	−0·02	**0·23***
PL						1·00	−0·01	0·12	−0·19	−0·05	−0·14	0·12	0·08	−0·15	0·04	0·07
PG							1·00	**0·99****	−0·10	−0·14	−0·06	**−0·21***	−0·02	0·08	0·07	−0·14
T-PL/PG								1·00	−0·12	−0·14	−0·08	−0·19	−0·01	−0·06	0·08	−0·13
T. proteol.									1·00	**0·38****	**0·33****	−0·04	−0·05	−0·03	**−0·21***	0·16
α-LA										1·00	**0·47****	0·04	0·03	−0·07	**−0·43****	−0·03
β-LG											1·00	−0·15	−0·14	0·10	**−0·73****	0·06
α-S_1CN												1·00	**−0·32****	0·12	−0·07	0·09
α-S_2CN													1·00	**−0·37****	−0·05	0·55
κ-CN														1·00	**−0·34****	−0·09
β-CN															1·00	−0·01
pH																1·00

Significance level of the observed correlations indicated; *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$. Abbreviations: T. prot. = total protein; T. WP = total whey protein; T. fat = total fat; FFA = free fatty acids; SCC = somatic cell count; PL = plasmin activity; PG = plasminogen derived activity; TA-PL/PG = total activity plasmin/plasminogen; T. proteol. = total proteolysis; α-LA = α-lactalbumin; β-LG = β-lactoglobulin; α-S_1CN = α- S_1 casein; α-S_2CN = α-S_2 casein; κ-CN = κ-casein; β-CN = β–casein

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total bacteria count could unfortunately not be performed on the sampling occasion for this study.

In conclusion, this study showed significant differences in the composition and proteolytic activity of bulk milk samples from farms using AMS and CMS, respectively. Despite a lower plasmin activity, milk from AMS herds showed higher total proteolysis and lower total casein and ß-casein fractions than milk from CMS herds. This could possibly be linked to an elevated SCC and higher proteolytic activity of cellular proteases in milk from AMS herds. Although the observed differences may be of limited practical significance, the proteolytic activity of raw milk needs further attention, affecting the stability and sensory attributes of many dairy products.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029917000140.

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