J. Dairy Sci. 107:6983–6993
https://doi.org/10.3168/jds.2023-24464
© 2024, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Milking system and diet forage type effect on milk quality of Italian Holstein-Friesian

Marica Simoni,[1] Rokia Temmar,[2] Massimo De Marchi,[2] Andrea Revello-Chion,[3] Marta Pozza,[2] Federico Righi,[1]* and Carmen L. Manuelian[4]

[1]Department of Veterinary Science, University of Parma, 43126 Parma, Italy
[2]Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro (PD), Italy
[3]Associazione Regionale Allevatori Piemonte, Laboratorio Analisi, 12100 Cuneo, Italy
[4]Group of Ruminant Research (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Spain

ABSTRACT

Moving from conventional (CMS) to automatic (AMS) milking systems could affect milk quality. Moreover, the type and preservation methods of the forages used in the TMR, such as alfalfa hay (HTMR) or corn silage (STMR) have been demonstrated to modify milk composition. Thus, this study investigated the effect of implementing AMS and different diet forage types on the quality of Italian Holstein-Friesian bulk milk. Milk samples (n = 168) were collected monthly from 21 commercial farms in northern Italy during a period of 8 mo. Farms were categorized into 4 groups according to their milking system (CMS vs. AMS) and diet forage type (HTMR vs. STMR). Milk quality data were analyzed through the mixed procedure for repeated measurement of SAS with the milking system, diet forage type, and sampling day as fixed effects. Milking through the AMS led to lower milk fat, freezing point, and β-LG A; longer coagulation time; and higher K content, pH, and β-LG B than CMS. Cows fed STMR produced milk with greater fat, protein, casein, Mg content, titratable acidity, and β-LG A, but with reduced curd firming time, freezing point, and β-LG B than those fed HTMR. In conclusion, milk quality is not only altered by the diet's forage type and characteristics but also by the milking system.

Key words: automated milking system, nutrition, milk quality, forages

INTRODUCTION

The implementation of automated milking systems (**AMS**), also known as robotic milking, equipped with auxiliary cooling, cleaning tools, and herd management software has steadily increased since their appearance in 1990 to support dairy farming (de Koning et al., 2003; De Marchi et al., 2017). The AMS allow voluntary access by lactating cows to the milking unit, which performs the complete milking and postmilking routine, while supplying an individual amount of concentrate based on parity, DIM, and milk yield (King et al., 2018). Although AMS reduces the time and the number of employees dedicated to milking-related activities (Bentley et al., 2013; Tse et al., 2018), it might affect feeding behavior and time budget, as well as rumen function and fermentation patterns. The latter relates to feedstuff consumption (Maekawa et al., 2002; Dijkstra et al., 2012), and cow behavior near the AMS (i.e., re-entering the AMS due to a failed milking visit and waiting near the exit gate), with consequences on milking efficiency and milk quality (Jacobs and Siegford, 2012). Moreover, AMS affects milk yield and quality due to an increase in milking frequency (Wagner-Storch and Palmer, 2003). Additionally, the freezing point (**FP**) tends to increase by shortening the intervals between milkings (Hogenboom et al., 2019). However, despite an increase in milk production and free fatty acids (**FA**) content (Wiking et al., 2006; De Marchi et al., 2017), several authors reported that AMS does not affect milk fat, protein, CN, lactose, Na, K, and Cl content (Abeni et al., 2005, 2008; Janštová et al., 2011; De Marchi et al., 2017). The effect of AMS on SCC is not consistent among studies. Some authors reported an increase in SCC of bulk milk (Rasmussen et al., 2002; Johansson et al., 2017), whereas others did not observe differences (Mollenhorst et al., 2011; Toušová et al., 2014; De Marchi et al., 2017). It is worth mentioning that a survey conducted in northeastern England found a higher incidence of clinical mastitis and a greater antibiotic administration associated with the use of AMS (Stergiadis et al., 2012).

In the key areas for cheese production of northern Italy, particularly those with Protected Designation of Origin

Received November 23, 2023.
Accepted April 16, 2024.
*Corresponding author: federico.righi@unipr.it

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

(**PDO**) cheeses such as Parmigiano Reggiano, Asiago, Grana Padano, and Provolone Valpadana, the prevalent diet's forage types fed in the farms are either alfalfa hay-based TMR (**HTMR**) or corn silage-based TMR (**STMR**). The difference between these diets lies in the family (grasses or legumes) and the preservation method (dry or ensiled) of the primary forage employed. Furthermore, these factors involve the use of diversified types of fibrous feed, concentrates, and additives to achieve the nutritional balance of the 2 types of diets. All these factors significantly affect the nutritional characteristics of the overall TMR diet type and produce differences in milk composition. To the best of our knowledge, only partial information is reported in the literature on the nutritional characteristics and related milk composition of these 2 diet types, whereas various studies have been performed individually on forage preservation method and family of forage. Contrasting results are reported on milk and cheese gross composition and flavor as affected by the dietary forage preservation method (Shingfield et al., 2005; Manzocchi et al., 2020; Serrapica et al., 2020; Van den Oever et al., 2021). Some authors observed that this factor can alter the profile of the nutrients produced and absorbed in the digestive tract (Huhtanen et al., 2010), thereby affecting the FA profile, volatile compounds, and sensory properties of milk and cheese (Van den Oever et al., 2021; Balivo et al., 2023). This is probably linked to the capacity of the ruminal microbiota and environment to modify FA supplied with the diet (Bionaz et al., 2020). It is widely recognized that grasses, compared with legumes, contain more NDF and ADF; lower amounts of CP, ash, and macro- and microminerals, especially Ca, K, and P; and their proteins are less soluble. Thus, grasses are considered forages of lower quality compared with legumes. Grass-based diets require greater quantities of cereals and byproducts to be balanced compared with legume-based diets. Performance differences attributable to grasses and legumes are often confused with differences in the NDF and ADF dietary content. When these 2 families of forages are included in diets, the comparison of the diets and their effect on milk yield and DMI must consider not only the dietary NDF content but also the forage NDF, digestibility, and particle size of the forages (Paulson et al., 2008).

Cheese production consortia of the main Italian PDO cheeses (Grana Padano, Asiago, Parmigiano Reggiano, and Provolone Valpadana) have expressed their concerns about the possible effect of robotic milking on milk quality, workability, and cheese production. Because these PDO cheeses are based on high-quality milk production (Parmigiano Reggiano, Council Regulation EC No. 510 [Council of the European Union, 2006] and Reg UE N. 794/2011 [European Commission, 2011b]; Asiago, Reg. UE N. 2020/1300 [European Commission, 2020]; Grana Padano, Reg UE N. 584, 2011 [European Commission, 2011a]; Provolone Valpadana, Reg. CE 1107/1996 [European Commission, 1996]), we hypothesize that the specific diet's forage types adopted in the main cheese production areas can significantly influence the quality of the milk together with the milking system.

Consequently, the study aimed to compare the quality of Holstein-Friesian bulk milk (1) obtained through AMS instead of CMS, and (2) from cows fed HTMR or STMR in northern Italy commercial dairy farms.

MATERIALS AND METHODS

An animal care and use statement was not required as this study dealt with milk samples collected directly from the bulk tank after milking. Initially, the study was intended to last 1 yr, but due to a second lockdown prompted by the COVID-19 pandemic during the autumn 2020, the study was interrupted for 4 mo. Thus, the study was conducted during an 8-mo interval, from June to October 2020 and from March to May 2021.

Description of Farms

Twenty-one commercial Holstein-Friesian herds (108 ± 63.5 cows, 177 ± 31 DIM, 2.4 ± 0.4 parity, mean ± SD) located across 3 northern Italian regions (i.e., Emilia Romagna, 8 farms; Veneto, 8 farms; and Piemonte, 5 farms) were enrolled on this study. All the farms selected had an internal herd production. Farms were selected and grouped to create uniform groups based on DIM, parity and number of cows, with calving distributed throughout the year. Moreover, farms with the same diet forage type and different milking systems were paired by geographical distance (within 15 km), obtaining similar environmental and soil conditions.

All the farms operated a TMR system and were enrolled according to milking system (CMS, n = 11, 116 ± 76 cows, 174 ± 33 DIM; AMS, n = 10, 103 ± 48 cows, 180 ± 29 DIM) and diet forage type (HTMR, n = 9, 121 ± 59 cows; 179 ± 33 DIM; STMR, n = 12, 98 ± 68 cows, 176 ± 29 DIM).

In farms with CMS, animals were milked twice a day, generally from 03:00 to 10:00 a.m. and from 03:00 to 10:00 p.m. Companies manufacturing the milking systems in the CMS farms were 9% BouMatic (Enne Effe Srl., Cremona, Italy), 9% TDM (Nutriservice S.r.l, Brescia, Italy), 9% Milkline (Milkline, Piacenza, Italy), 18% Tecnozoo (Tecnozoo Impianti S.r.l., Zelo Buon Persico, LO, Italy), 18% GEA (GEA Group Aktiengesellschaft, Dusseldorf, Germany), and 36% DeLaval (DeLaval S.p.a., San Donato Milanese, MI, Italy). In farms with AMS, the milking robot were 70% Lely Astronaut (Lely, Colturano, MI), 20% VMS (DeLaval S.p.a., San Donato

Milanese, MI), and 10% Fullwood Merlin (Fullwood, Ellesmere, UK). The average milking frequency for the AMS was 2.67 ± 0.31. Farms equipped with AMS supplied 3.44 ± 0.87 kg/d of feedstuff in the milking robot.

Farms were categorized as follows: (1) CMS and HTMR, n = 5 farms; (2) CMS and STMR, n = 6 farms; (3) AMS and HTMR, n = 4 farms; and (4) AMS and STMR, n = 6 farms. The HTMR and the STMR differed in the main forage family included in the diet, its method of preservation, amount and typology of concentrate added to balance the diet. The HTMR (NE_L: 1.93 ± 0.096 Mcal/kg, mean ± SD) was mainly composed of alfalfa hay (leguminous) from different cuts, followed by a graminaceous mixed hay. On average, the forage-to-concentrate ratio of the HTMR was around 0.7:1 (44% forage and 56% concentrate; according to Mertens (2009) corn silage and earlage were considered only 50% as forages, while graminaceous silage as wheat or triticale silage were considered as 70% forages). The forages adopted in the HTMR were harvested within the same territory adhering to PDO cheeses regulations. Most of the hay was sun-dried, and a small proportion of farmers used the forage dehydration. The STMR (NE_L: 2.09 ± 0.092 Mcal/kg, mean ± SD) consisted mainly of corn silage (grasses) followed by other ensiled forages (e.g., earlage, alfalfa silage, wheat, or ryegrass silage). On average, the forage-to-concentrate ratio of the STMR was around 1.5:1 (42% forage and 58% concentrate). All the silages included in the diets were produced, harvested, and stored on the farm. Corn silages were harvested using shredlage and stored in concrete bunks, this storing system was adopted also for the earlage, while all the other silages were stored in wrapped bales. In each farm, a composite TMR sample and a sample of the feedstuff provided in the AMS were collected monthly from 10 subsamples taken along the feeding line, and chemically analyzed. In brief, DM, ashes, ether extract (**EE**), and starch content were determined following European Commission methods (Regulation No. 152/2009; European Commission, 2009). Fiber fractions (heat-stable amylase for NDF [**aNDF**], ADF, and lignin) were analyzed sequentially using aNDF determination without the use of sodium sulfite (Robertson and Van Soest, 1981). The undigestible NDF (**uNDF**) content was determined through a 240-h in vitro fermentation according to Raffrenato et al. (2018). The CP content was quantified by Dumatherm (Gerhardt GmbH & Co., Königswinter, Germany) as described by Mihaljev et al. (2015). The NSC were calculated by the difference between 100 and the sum of ash, EE, CP, and aNDF. The average feed chemical composition is displayed in Table 1.

Bulk Milk Sampling and Milk Quality Analyses

A total of 168 bulk milk samples were collected throughout the study. Bulk milk samples were collected monthly from the tank after the morning and evening milking and mixed to be representative of the herd's 24-h milk production. Each sample was then split into 2 aliquots in 50-mL tubes containing preservative (bronopol,

Table 1. Dietary formula and chemical composition[1] (mean and SD) of the alfalfa hay-based TMR (HTMR) and corn silage-based TMR (STMR) fed in the farms enrolled in the study

Item	n	HTMR	n	STMR
Dietary formula, % DM				
Corn silage			12	30.3 (3.7)
Corn earlage			12	6.4 (4.8)
Graminaceous silage			12	2.3 (2.8)
Alfalfa silage			12	4.4 (4.79)
Alfalfa hay	9	28.0 (5.0)	12	7.8 (4.5)
Graminaceous hay	9	13.6 (5.0)	12	8.6 (7.1)
Straw	9	0.1 (0.2)	12	0.4 (0.5)
Cereals and feedstuffs	9	58.3 (4.3)	12	39.8 (3.5)
Forage,[2] % DM	9	44.4 (3.1)	12	41.7 (4.3)
Concentrate, % DM	9	55.6 (3.1)	12	58.3 (4.3)
Chemical composition, % DM				
CP	72	14.60 (1.49)	96	15.27 (2.17)
aNDF	72	40.51 (4.97)	96	36.71 (4.95)
ADF	72	24.18 (3.66)	96	20.92 (2.37)
uNDF	72	14.08 (3.23)	96	11.61 (2.72)
Lignin	72	4.72 (1.23)	96	4.00 (1.42)
Starch	72	19.32 (3.65)	96	24.50 (3.55)
Nonstructural carbohydrate	36	36.03 (6.17)	45	39.28 (5.62)
Ether extract	36	2.74 (0.66)	45	3.80 (1.04)
Ash	72	8.26 (1.89)	96	7.12 (1.09)

[1]aNDF = NDF treated with a heat-stable α-amylase; uNDF = undigestible NDF.

[2]Corn silage was considered 50% forage; other grass silages were considered 70% forage, according to Mertens (2009).

2-bromo-2-nitropropan-1,3-diol; D&F Inc., Dublin, CA) and transported to the laboratory of the regional breeder association for the milk quality analyses. According to the International Committee for Animal Recording recommendations, bulk milk composition (fat, protein, CN, lactose percentages, and FP) and urea were determined using the MilkoScan 7 (Foss Electric A/S, Hillerød, Denmark). Moreover, SCC was determined by flow cytometers with Fossomatic FC (Foss Electric A/S), and values expressed as cells/μL were transformed into SCS through the formula SCS = 3 + $\log_2$ (SCC/100), as suggested in Wiggans and Shook (1987).

The pH was evaluated using a potentiometric pH meter (Mettler Delta 345; Mettler Toledo SpA, Novate Milanese, Italy). The titratable acidy was determined by titrating milk with a 0.25 N NaOH solution until a pH of 8.30 using a Crison Compact D meter (Crison Instruments SA, Alella, Spain) and expressed as Soxhlet-Henkel degrees (Penasa et al., 2016). The content of the major minerals in bulk milk was quantified using inductively coupled plasma-optical emission spectrometry (**ICP-OES**) Arcos EOP (SPECTRO Analytical Instruments GmbH, Kleve, Germany) following the AOAC International method 2013.06 (AOAC International, 2016). The calibration solutions for each mineral were prepared from single-element solutions (Inorganic Ventures, Christiansburg, VA) in a concentration range between 0 and 100 mg/L. Mineral contents were expressed in milligrams per kilogram of milk.

The assessment of milk coagulation traits was performed through lactodynamographic analysis (MaPe System, Firenze, Italy) according to the protocol reported by Vigolo et al. (2022) using 200 μL of commercial calf rennet solution (Naturen Plus 215, Chr Hansen, Hørsholm, Denmark) diluted with distilled water (1.2:100, vol/vol). The milk coagulation traits obtained were rennet coagulation time (**RCT**), time to a curd firmness of 20 mm ($\mathbf{k_{20}}$), and curd firmness at 30 min after coagulant addition ($\mathbf{a_{30}}$). The quantification of CN and whey protein fractions was performed on raw milk by using the HPLC station Agilent 1260 Infinity II LC (Agilent Technologies, Santa Clara, CA) equipped with a quaternary pump (Agilent 1260 Infinity II, G7111B), a diode array detector (Agilent 1260 Infinity II, G7115A), a column thermostat (Agilent 1260 Infinity II, G7116A), and an autosampler (Agilent 1260 Infinity II, G7129A) following the method described in Franzoi et al. (2022).

Statistical Analysis

The sample size was calculated with G*Power software v. 3.1.9.6 (Faul et al., 2007; 2009; Heinrich Heine Universität Düsseldorf, Germany). The normality of the data was verified through the UNIVARIATE procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC), and the outliers identified were treated as missing values. Outliers were <4.3% for AMS and <10.9% for CMS. Outliers were <10.3% for STMR and <2.8% for HTMR. Sources of variation of milk traits were analyzed using the MIXED procedure with repeated measurement of SAS v. 9.4. The model considered milking system, diet forage type, and the sampling day as fixed effects and farm nested within region as random effect. Results were expressed as LSM and separated using Tukey's multiple comparison test. The LSM for the sampling day are not shown, nor discussed. Significance was declared at $P < 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Milking System and Milk Quality

To date, there is no clear consensus on the effect of the milking system on milk gross composition. Although we observed a greater fat content in milk from CMS than AMS (Table 2), most of the studies did not report an effect of the milking systems on milk fat, protein, CN, and lactose content (Janštová et al., 2011; Innocente and Biasutti, 2013; De Marchi et al., 2017). In contrast, Klungel et al. (2000) and Toušová et al. (2014) found greater fat and protein content in bulk milk from AMS than CMS, whereas Johansson et al. (2017) reported a lower protein content. Nevertheless, the decrease in fat content in the milk of cows milked with the AMS could be related to a possible increase in milk yield due to the higher milking frequency that is usually associated with this milking system (Klungel et al., 2000; Abeni et al., 2005).

Our results regarding the SCS (Table 2) agreed with Toušová et al. (2014), who also found similar results when comparing AMS with CMS in a Fleckvieh cow herd. However, other authors have reported an initial increase with a subsequent decrease when switching from CMS to AMS (Rasmussen et al., 2002; Johansson et al., 2017). Furthermore, Janštová et al. (2011) reported lower values when Holstein cows were milked with an AMS. Therefore, the lack of significant differences in SCS we observed could indicate that the cows in the selected farms were well adapted to the AMS.

The similarity of the urea concentration between milking systems (Table 2) is consistent with findings from Stergiadis et al. (2012), who compared milk from Holstein-Friesian cows milked with CMS or AMS. In contrast, Toušová et al. (2014) reported greater urea concentration with AMS (+21.8%) explained by the shorter intervals between milkings (Nielsen et al., 2005).

Few studies have evaluated differences in milk mineral content between CMS and AMS, despite their important role in the colloidal stability of the CN micelle

Table 2. Least squares means ± SE of gross composition, SCS, urea, and minerals from cow bulk milk based on the milking system[1] and diet forage type[2]

	Milking system					Diet forage type				
Item	n	CMS	n	AMS	*P*-value	n	HTMR	n	STMR	*P*-value
Fat, %	82	3.78 ± 0.04	76	3.60 ± 0.05	0.005	70	3.57 ± 0.05	88	3.81 ± 0.04	<0.001
Protein, %	81	3.36 ± 0.02	76	3.35 ± 0.02	0.598	70	3.34 ± 0.02	87	3.38 ± 0.02	0.075
Casein, %	82	2.65 ± 0.02	76	2.63 ± 0.02	0.354	70	2.62 ± 0.02	88	2.66 ± 0.02	0.059
Lactose, %	82	4.84 ± 0.01	75	4.82 ± 0.01	0.186	70	4.83 ± 0.01	87	4.83 ± 0.01	0.802
SCS	82	5.22 ± 0.04	76	5.27 ± 0.04	0.413	70	5.26 ± 0.04	88	5.24 ± 0.04	0.715
Urea, mg/dL	82	23.8 ± 1.14	76	25.5 ± 1.14	0.099	70	24.5 ± 1.18	88	24.8 ± 1.12	0.814
Ca, mg/kg	44	1,050 ± 9.34	40	1,026 ± 9.82	0.073	36	1,026 ± 10.3	48	1,050 ± 8.88	0.086
K, mg/kg	44	1,730 ± 14.6	40	1,764 ± 15.0	0.012	36	1,756 ± 15.5	48	1,738 ± 14.4	0.184
Mg, mg/kg	44	114 ± 0.83	40	112 ± 0.88	0.069	36	111 ± 0.92	48	114 ± 0.80	0.029
Na, mg/kg	44	404 ± 6.65	40	400 ± 6.94	0.742	36	407 ± 7.34	48	396 ± 6.28	0.255
P, mg/kg	44	1,069 ± 9.19	40	1,062 ± 9.50	0.478	36	1,056 ± 9.92	48	1,073 ± 8.96	0.169

[1]CMS = conventional milking parlor; AMS = automatic milking system.

[2]HTMR = hay-based TMR; STMR = silage-based TMR.

(Cashman, 2011). Variations in the milk mineral content could indicate an altered permeability of the mammary epithelia because the opening of tight junctions facilitates the transfer of minerals to blood (Hogenboom et al., 2019). Therefore, the decrease in milk mineral content when implementing an AMS could also be an indicator of mammary gland physical trauma. However, in the present study, we only observed a greater K content in AMS than CMS (Table 2), although Abeni et al. (2008) reported no effect of the milking system on Na, K, and Cl content.

In agreement with De Marchi et al. (2017), our results showed a greater pH in AMS than CMS (Table 3). In contrast, Priyashantha et al. (2021) reported unaffected pH. Milk FP is relatively constant, because it depends on the osmotic equilibrium between blood and milk (Hogenboom et al., 2019), and is a good indicator of milk adulteration with water. The observed greater FP in AMS than CMS (Table 3) agreed with Klungel et al. (2000) and de Koning et al. (2003) results. These findings could be explained by the increase in cleaning frequency and rinsing of the system, which adds some residual water to the milk (Rasmussen et al., 2002).

Milk proteins have gained interest in the dairy industry mainly for their role in human nutrition and cheese production. In our study, the milking system had a limited effect on bulk milk protein fraction, with an increase in β-LG B and a decrease in β-LG A in AMS compared with CMS (Table 4). However, this result should not affect cheese yield, because the latter is positively correlated with β-CN content and negatively associated with β-LG content (Cipolat-Gotet et al., 2020).

Diet Forage Type and Milk Quality

Contrasting results regarding the effects of the forage family on intake, milk yield, and quality are reported in the literature (Paulson et al., 2008; Steinshamn, 2010; Dewhurst et al., 2003). Grass-based diets generally require a lower forage-to-concentrate ratio and thus, higher energy intake from concentrate, especially when adjusted to equal NDF levels (Paulson et al., 2008). A recent meta-analysis revealed that legume-based diets allow higher DMI and milk yield but result in decreased milk protein and fat content (Johansen et al., 2018). However, the effect of forage family on cow performance is influ-

Table 3. Least squares means ± SE of technological traits[1] from cow bulk milk based on the milking system[2] and diet forage[3] type

	Milking system					Diet forage type				
Item	n	CMS	n	AMS	*P*-value	n	HTMR	n	STMR	*P*-value
pH	87	6.73 ± 0.02	78	6.75 ± 0.02	0.017	71	6.74 ± 0.02	94	6.74 ± 0.02	0.542
TA, °SH/50 mL	87	3.13 ± 0.05	78	3.04 ± 0.05	0.074	71	3.02 ± 0.05	94	3.15 ± 0.05	0.013
RCT, min	85	21.1 ± 1.30	74	22.4 ± 1.30	0.086	68	22.2 ± 1.31	91	21.4 ± 1.28	0.301
k_{20}, min	52	7.53 ± 0.21	41	7.81 ± 0.23	0.364	38	8.11 ± 0.25	55	7.24 ± 0.20	0.008
a_{30}, mm	86	19.0 ± 2.90	73	16.51 ± 2.91	0.173	68	16.58 ± 2.96	91	18.93 ± 2.88	0.215
Freezing point, °C	82	−0.519 ± −0.684	76	−0.516 ± −0.690	<0.001	70	−0.517 ± −0.714	88	−0.518 ± −0.669	0.004

[1]TA = titratable acidity; °SH = Soxhlet-Henkel degree; RCT = rennet coagulation time; a_{30} = curd firmness; k_{20} = curd firming time.

[2]CMS = conventional milking parlor; AMS = automatic milking system.

[3]HTMR = hay-based TMR; STMR = silage-based TMR.

Table 4. Least squares means ± SE of protein fraction from cow bulk milk based on the milking system[1] and diet forage type[2]

Item	Milking system					Diet forage type				
	n	CMS	n	AMS	*P*-value	n	HTMR	n	STMR	*P*-value
α_{S1}-CN	63	8.62 ± 0.07	57	8.53 ± 0.08	0.422	51	8.53 ± 0.08	69	8.63 ± 0.07	0.330
α_{S1}-CN-17	74	10.2 ± 0.08	67	10.2 ± 0.08	0.656	60	10.1 ± 0.08	81	10.2 ± 0.07	0.465
α_{S2}-CN	63	2.41 ± 0.06	57	2.38 ± 0.06	0.590	51	2.36 ± 0.07	69	2.43 ± 0.06	0.301
α_{S2}-CN-41	74	3.70 ± 0.07	67	3.70 ± 0.07	0.999	60	3.65 ± 0.08	81	3.74 ± 0.07	0.290
α_{S}-LA	74	1.01 ± 0.08	67	1.04 ± 0.08	0.181	60	1.03 ± 0.08	81	1.02 ± 0.08	0.891
β-CN	74	12.3 ± 0.09	67	12.2 ± 0.09	0.919	60	12.2 ± 0.10	81	12.3 ± 0.09	0.253
β-CNa1	53	4.33 ± 0.26	47	4.36 ± 0.27	0.900	43	4.22 ± 0.28	57	4.47 ± 0.28	0.335
β-CNa2	53	7.19 ± 0.20	47	7.04 ± 0.21	0.510	43	7.02 ± 0.22	57	7.21 ± 0.19	0.425
β-CNb	37	0.72 ± 0.08	36	0.69 ± 0.08	0.547	39	0.69 ± 0.08	34	0.72 ± 0.08	0.557
β-LG A	74	2.61 ± 0.09	67	2.42 ± 0.09	0.008	60	2.39 ± 0.09	81	2.64 ± 0.09	<0.001
β-LG B	74	1.25 ± 0.08	67	1.35 ± 0.08	0.034	60	1.38 ± 0.08	81	1.22 ± 0.08	0.002
κ-CN	63	5.22 ± 0.11	57	5.22 ± 0.11	0.951	51	5.16 ± 0.11	69	5.28 ± 0.10	0.247
κ-CN-8	74	5.75 ± 0.10	67	5.79 ± 0.10	0.704	60	5.73 ± 0.11	81	5.81 ± 0.10	0.406
κ-CN-ae	53	3.83 ± 0.13	47	3.76 ± 0.14	0.666	43	3.77 ± 0.14	57	3.82 ± 0.13	0.758
κ-CN-b	53	1.16 ± 0.07	47	1.25 ± 0.07	0.364	43	1.18 ± 0.08	57	1.23 ± 0.06	0.560

[1]CMS = conventional milking parlor; AMS = automatic milking system.

[2]HTMR = hay-based TMR; STMR = silage-based TMR.

enced by several factors such as forage species (Salawu et al., 2002), forage maturity (and therefore, digestibility), leaf-to-steam ratio, and cell wall structure (Paulson et al., 2008). Moreover, the quality and composition of different forages depend also on the forage preservation methods applied. In fact, ensiling forages can be more easily harvested at the optimal stage of maturity, and are less exposed to environmental conditions and to the loss of nutrients from harvest to storage (Coblentz and Akins, 2018; Grant and Ferraretto, 2018; Wilkinson and Rinne, 2018). Therefore, in general, silage-based diets allow higher productivity due to higher forage digestibility and DMI (Grant and Ferraretto, 2018). Differences have been studied between grass and legume silages, the latter allowing greater intake (Dewhurst et al., 2003; Steinshamn, 2010), which Salawu et al. (2002) attributed to the higher rate of fermentation and passage of legumes. In the present study, 2 diet forage types with different forage bases were compared; they differed in forage family and preservation method of the primary forage included in the diet, grass-to-legume ratio, and amount of concentrate added.

The formula and chemical composition of the diets used in the present study (Table 1) is representative of the geographical area where the study was conducted (Comino et al., 2015; Manuelian et al., 2021; Simoni et al., 2021; Esposito et al., 2024). These studies reported diets contained varying proportions of corn silage (24%–31.9% DM) or alfalfa hay as base forage (13.2%–50.0% DM). The chemical composition of these diets varied with CP (13.7%–18.1% DM), NDF (30.5%–39.9% DM), starch (19.8%–27.2% DM), EE (2.3%–4.7% DM), NFC (33.1%–41.2% DM), and ash (6.37%–10.4% DM) content. Several studies have shown that high-producing lactating cattle can maintain high milk yield and milk protein production, even when they are fed relatively low-protein diets, typically containing 14% to 15.5% CP content (Hofherr, 2010; Higgs et al., 2012), if adequate rumen carbohydrate fermentation is achieved. Our diets fall in the aforementioned range, and the aNDF content of all the diets exceeded recommended level by Chase and Overton (2004) of 33% DM.

The lower CP and higher fiber content of the diets HTMR compared with STMR in the present study (Table 1) could be attributed to alfalfa leaves loss during baling due to structural fragility of the leaves (Salawu et al., 2002), and to the lower amount of dietary concentrate in the diet. Dietary recommendations commonly advise against feeding more than 8% DM of fat in lactating cattle diets (Erickson and Kalscheur, 2020). High-producing cows can receive a diet of up to 5% to 6% of fat (Bionaz et al., 2020). In a typical TMR containing no supplemental fat, the fat content is usually in the range of ~3% to 4% (Erickson and Kalscheur, 2020). This range is higher than the values found for the HTMR, in which no supplemental fat was included in the ration due to PDO cheese production regulations (Council Regulation EC No. 510, Council of the European Union, 2006; Reg UE N. 794/2011, European Commission, 2011b; and Reg. UE N. 2020/1300, European Commission, 2020). In line with our results, Brito and Broderick (2006) reported a greater starch content and ruminal fermentable carbohydrates of corn silage- compared with hay-based diet. In fact, as the corn becomes more mature the ruminal starch availability is reduced as happens in corn meal compared with starch included in corn silage. In the present study (Table 1) the starch content of the HTMR falls below the recommended range for dietary starch, which typically ranges from 23%

to 30% DM (Grant, 2005), thus providing a less energetic diet. On the other hand, the STMR had a higher NSC content of which 62.4% was starch, particularly from corn silage and earlage (32% of the total starch), indicating more digestible starch compared with the processed cereals included in feedstuffs. The highest starch content is known to increase protozoa (Sniffen and Formigoni, 2006) and ruminal propionate production, which can be efficiently converted to glucose, potentially increasing mammary casein synthesis (Mordenti et al., 2017). In our study, a trend for higher milk protein and casein was observed for STMR. Generally, silage provides fermentation end products such as acetic acid (1.0%–3.0% DM) and ethanol (0.5%–1.5% DM; Kung et al., 2018). The absorbed acetic acid from silage by the rumen is used as an energy source or it is incorporated into milk or body fat (Kung et al., 2018). Silage ethanol can be directly absorbed by the rumen wall or transformed to acetic acid in the rumen and finally converted to milk fat or is available for body metabolism or growth (Kung et al., 2018). Thus, the availability of the above-mentioned substances, together with a more energetic diet may explain the greater milk fat content observed in the present study from cows fed STMR instead of HTMR (Table 2).

Silages yield a significant amount of soluble N, accounting for ~55% to 60% of the total N. This soluble N is a product of the action of bacterial proteases during the fermentation process, further enhancing the nutritional value of silage-based diets, as noted in the study by Kung et al. (2018). Opposite evidence was imputed by Huhtanen et al. (2003) to the extensive fermentation of the silages leading to higher ammonia nitrogen content whose consequence is a reduction in DMI and microbial protein synthesis. The higher NFC and starch content of the STMR combined with the higher forage digestibility led to a higher microbial protein synthesis, which is beneficial for milk protein synthesis (Rosmalia et al., 2022). The greater milk fat, with the trend observed for higher protein and casein content and higher ECM (data not shown) in STMR compared with HTMR, suggests a more intense ruminal metabolism with enhanced VFA production, increased microbial protein synthesis, and an overall higher nutrient supply to the small intestine (Mordenti et al., 2017). The observed effects of the diet forage types on milk quality parameters in Table 2 are partially in agreement with the available literature. No effect of the forage preservation method was found on milk yield and milk fat content by several authors (Serrapica et al., 2020; Manzocchi et al., 2021). Lower milk fat, together with higher milk yield was observed when legume silages were fed instead of grass silages by Steinshamn (2010). On the other side, Dewhurst et al. (2003) observed a higher yield of milk, but also higher milk fat, protein, and lactose when comparing legume silages to grass silages. Including a higher proportion of alfalfa, the HTMR diets generally could have a higher soluble protein content. Moreover, the HTMR in the present study are characterized by higher uNDF and lower dietary starch content, providing less energy for microbial growth and rumen function.

Among mineral content in milk (Table 2), only Mg showed a significant difference between both groups, revealing greater content when cows were fed with STMR instead of HTMR (Table 2). A third of milk Mg is associated with casein (Gaucheron, 2005), which could explain the greater Mg content in milk from STMR than HTMR. Moreover, it has been also reported a higher absorption of silage minerals compared with alfalfa hay (Martz et al., 1990), and that the concentration of Ca, Mg, and Zn have also been shown to increase when adopting the silage conservation method as opposed to the drying hay process (Schlegel et al., 2018). This increase can be attributed to the release of gases during fermentation and the volatilization of certain compounds throughout the fermentation process (Schlegel et al., 2018). Despite the higher mineral content of the alfalfa forage, the relative bioavailability in hay is potentially low because they are often produced from plants harvested at late stages of maturity (Spears, 1994). The latter author reported a markedly decreased content in several minerals, especially P, with increasing maturity of forages (Spears, 1994). Moreover, harvesting process exert a high impact on macro- and micromineral concentrations due to mechanical losses (Schlegel et al., 2018). On the other hand, Manzocchi et al. (2021) reported no effect of the preservation method on milk mineral content with exception for P content, which was greater in cow milk fed a ryegrass hay-based diet.

The effects of the diet's forage type on milk technological traits observed in the present study (Table 3) are partially in agreement with Manzocchi et al. (2020), who found similar milk acidity, RCT, k_{20}, and a_{30} after feeding diets based on ryegrass hay, grassland, or corn silage-based to dairy cattle. The increased TA in the STMR milk may be due to the combination of numerically higher protein and P, and higher Mg content in the milk, according to Schmitt et al. (2014). The higher k_{20} found in HTMR milk may be also a consequence of the lower fat content of the milk from those farms (Table 2). Concerning the RCT, which was similar between diets, our study is partially in contrast with the results of Coulon et al. (1995), who reported lower RCT in the milk from corn silage-fed cows. From a practical standpoint, the increased fat and Mg content of the milk of the STMR group, along with the shorter curd firming time, suggest a potential for higher cheese yield and shorter cheese-making process. Which in turn, means enhanced production efficiency and income.

Milk protein fractions were unaffected by the diet's forage type, except for β-LG A and β-LG B (Table 4). The β-LG is a whey protein of the lipocalins family (Kontopidis et al., 2004), which acts as a nano-transporter for several molecules (e.g., FA, retinoid species, vitamin A and D, cholesterol; Edwards and Jameson, 2014). Varlamova and Zaripov (2020) reported that milk from cows carrying the β-LG gene in the B allele gave the highest yield and a denser rennet clot, which resulted in shorter coagulation time. In our study, the highest β-LG B was found in milk from HTMR fed cows which is in contrast with the mentioned research results. However, it should be noted here that our findings could have been affected in this case by the genomic divergence within the Italian Holstein breed for the milk protein fractions, related to the use of this breed in different dairy production contexts, as highlighted by Ablondi et al. (2021). Thus, an imbalanced distribution of the different β-LG genotypes in the studied herds could have biased our results for this phenotypic trait. For instance, the regulation of the Parmigiano Reggiano cheese tends to enhance the internal recruitment of replacement heifers. Moreover, part of these differences may also be affected by the herd's variation in the stage of lactation.

CONCLUSIONS

The results of the present study highlight the effect of the implementation of AMS on milk quality, which leads to a lower milk fat and β-LG A, and greater K content, pH, FP, and β-LG B. On the other hand, the use of corn silage instead of alfalfa hay as the main dietary forage and the consequent modifications of the diet ingredients yielded milk with a greater fat, Mg, titratable acidity, and β-LG A, and lower k_{20}, FP, and β-LG B. Thus, milk composition differences are mirroring both diet forage type and milking system.

NOTES

This work has been supported by AGER 2 Project, grant n° 20171153 - INNOVAMILK. The research was funded by Fondazione Cariplo, Milan, Italy. We thank all the farmers who participated in the project. C. L. Manuelian is currently a postdoctoral researcher funded with a Maria Zambrano grant from the Spanish Ministry of Universities, Madrid, Spain (funded by European Union-Next Generation EU; MZ2021-86). The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The data presented in this study are available free of charge for any user upon reasonable request from the corresponding author. This study used milk samples collected directly from the bulk tank after milking; no human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: a_{30} = curd firmness; AMS = automated milking systems; CMS = conventional milking system; EE = ether extract; FA = fatty acids; FP = freezing point; HTMR = alfalfa hay-based TMR; k_{20} = curd firming time; PDO = Protected Designation of Origin; RCT = rennet coagulation time; °SH = Soxhlet-Henkel degree; STMR = corn silage-based TMR; TA = titratable acidity.

REFERENCES

Abeni, F., L. Degano, F. Calza, R. Giangiacomo, and G. Pirlo. 2005. Milk quality and automatic milking: Fat globule size, natural creaming, and lipolysis. J. Dairy Sci. 88:3519–3529. https://doi.org/10.3168/jds.S0022-0302(05)73037-X.

Abeni, F., M. G. Terzano, M. Speroni, L. Migliorati, M. Capelletti, F. Calza, L. Bianchi, and G. Pirlo. 2008. 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. J. Dairy Sci. 91:3372–3384. https://doi.org/10.3168/jds.2008-1039.

Ablondi, M., M. Malacarne, C. Cipolat-Gotet, J. T. van Kaam, A. Sabbioni, and A. Summer. 2021. Genome-wide scan reveals genetic divergence in Italian Holstein cows bred within PDO cheese production chains. Sci. Rep. 11:12601. https://doi.org/10.1038/s41598-021-92168-1.

AOAC International. 2016. Official Methods of Analysis. 20th ed. AOAC International.

Balivo, A., R. Sacchi, and A. Genovese. 2023. The Noble Method in the dairy sector as a sustainable production system to improve the nutritional composition of dairy products: A review. Int. J. Dairy Technol. 76:313–328. https://doi.org/10.1111/1471-0307.12941.

Bentley, J. A., L. F. Tranel, L. L. Timms, and K. Schulte. 2013. Automatic milking systems (AMS)—Producer surveys. Iowa State University Animal Industry Report 10(1). https://doi.org/10.31274/ans_air-180814-739.

Bionaz, M., E. Vargas-Bello-Pérez, and S. Busato. 2020. Advances in fatty acids nutrition in dairy cows: From gut to cells and effects on performance. J. Anim. Sci. Biotechnol. 11:110. https://doi.org/10.1186/s40104-020-00512-8.

Brito, A. F., and G. A. Broderick. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:3924–3938. https://doi.org/10.3168/jds.S0022-0302(06)72435-3.

Cashman, K. D. 2011. Milk macroelements: Nutritional significance. Pages 923–929 in Encyclopedia of Dairy Science. J. W. Fuquay, P. F. Fox, and P. L. H. McSweeney, ed. https://doi.org/10.1016/B978-0-12-818766-1.10170-9.

Chase, L. E., and T. R. Overton. 2004. Guidelines for interpreting CPM_Dairy 305 Beta outputs for lactating dairy cows. Penn State Dairy Cattle Nutr. 2004:48–51.

Cipolat-Gotet, C., M. Malacarne, A. Summer, A. Cecchinato, and G. Bittante. 2020. Modeling weight loss of cheese during ripening and the influence of dairy system, parity, stage of lactation, and composition of processed milk. J. Dairy Sci. 103:6843–6857. https://doi.org/10.3168/jds.2019-17829.

Coblentz, W. K., and M. S. Akins. 2018. Silage review: Recent advances and future technologies for baled silages. J. Dairy Sci. 101:4075–4092. https://doi.org/10.3168/jds.2017-13708.

Comino, L., F. Righi, M. Coppa, A. Quarantelli, E. Tabacco, and G. Borreani. 2015. Relationships among early lactation milk fat depression,

cattle productivity and fatty acid composition on intensive dairy farms in Northern Italy. Ital. J. Anim. Sci. 14:3656. https://doi.org/10.4081/ijas.2015.3656.
Coulon, J. B., P. Pradel, and I. Verdier. 1995. Effect of forage type on milk yield, chemical composition and clotting properties of milk. Lait 75:513–521. https://doi.org/10.1051/lait:1995640.
Council of the European Union. 2006. Council regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. Off. J. Eur. Union 50:12–25.
de Koning, K., B. Slaghuis, and Y. van der Vorst. 2003. Robotic milking and milk quality: Effects on bacterial counts, somatic cell counts, freezing point and free fatty acids. Ital. J. Anim. Sci. 2:291–299. https://doi.org/10.4081/ijas.2003.291.
De Marchi, M., M. Penasa, and M. Cassandro. 2017. Comparison between automatic and conventional milking systems for milk coagulation properties and fatty acid composition in commercial dairy herds. Ital. J. Anim. Sci. 16:363–370. https://doi.org/10.1080/1828051X.2017.1292412.
Dewhurst, R. J., W. J. Fisher, J. K. Tweed, and R. J. Wilkins. 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86:2598–2611. https://doi.org/10.3168/jds.S0022-0302(03)73855-7.
Dijkstra, J., J. L. Ellis, E. Kebreab, A. B. Strathe, S. López, J. France, and A. Bannink. 2012. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed Sci. Technol. 172:22–33. https://doi.org/10.1016/j.anifeedsci.2011.12.005.
Edwards, P. J. B., and G. B. Jameson. 2014. Chapter 7: Structure and stability of whey proteins. Pages 201–242 in Milk Proteins from Expression to Food. 2nd ed. H. Singh, M. Bolnad, and A. Thompson, ed. Academic Press, San Diego, CA. https://doi.org/10.1016/B978-0-12-405171-3.00007-6.
Erickson, P. S., and K. F. Kalscheur. 2020. Chapter 9: Nutrition and feeding of dairy cattle. Pages 157–180 in Animal Agriculture: Sustainability, Challenges and Innovations. F. W. Bazer, G. C. Lamb, and G. Wu, ed. Academic Press, San Diego, CA. https://doi.org/10.1016/B978-0-12-817052-6.00009-4.
Esposito, G., M. Simoni, L. Quaini, D. Bignamini, A. Costa, and F. Righi. 2024. Impact of pre-partum nutraceutical or monensin intraruminal boluses on colostrum quality and Holstein dairy cows' performance: Exploratory field study. Ital. J. Anim. Sci. 23:479–491. https://doi.org/10.1080/1828051X.2024.2325048.
European Commission. 1996. Regolamento (CE) N. 1107/96 della Commissione del 12 giugno 1996 Off. J. Eur. Union. https://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:01996R1107-20081120.
European Commission. 2009. Commission regulation (EC) no. 152/2009 of 27 January 2009. Laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union. 54:1–130.
European Commission. 2011a. Regolamento di esecuzione (UE) n. 584/2011 della Commissione, del 17 giugno 2011. Off. J. Eur. Union L 160:65 https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:160:0065:0070:IT:PDF.
European Commission. 2011b. Regolamento (UE) n. 794/2011 della Commissione, dell' 8 agosto 2011. Off. J. Eur. L 204. 9 August 2011. CELEX 0794(number: 32011R). https://doi.org/10.3000/17252555.L_2011.204.eng.
European Commission. 2020. Regolamento di esecuzione (UE) 2020/1300 della Commissione dell'11 settembre 2020. Off. J. Eur. Union L 304:18 http://data.europa.eu/eli/reg_impl/2020/1300/oj.
Faul, F., E. Erdfelder, A. Buchner, and A. G. Lang. 2009. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav. Res. Methods 41:1149–1160. https://doi.org/10.3758/BRM.41.4.1149.
Faul, F., E. Erdfelder, A. G. Lang, and A. Buchner. 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39:175–191. https://doi.org/10.3758/BF03193146.
Franzoi, M., A. Costa, V. Vigolo, M. Penasa, and M. De Marchi. 2022. Effect of pasteurization on coagulation properties of bovine milk and the role of major composition traits and protein fractions. J. Food Compos. Anal. 114:104808. https://doi.org/10.1016/j.jfca.2022.104808.
Gaucheron, F. 2005. The minerals of milk. Reprod. Nutr. Dev. 45:473–483. https://doi.org/10.1051/rnd:2005030.
Grant, R. 2005. Optimizing starch concentrations in dairy rations. Pages 73–79 in WH Miner Agricultural Research Institute Tri-State Dairy Nutrition Conference, Fort Wayne, IN. The Ohio State University, Columbus, OH.
Grant, R. J., and L. F. Ferraretto. 2018. Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. J. Dairy Sci. 101:4111–4121. https://doi.org/10.3168/jds.2017-13729.
Higgs, R. J., L. E. Chase, and M. E. Van Amburgh. 2012. Case study: Application and evaluation of the Cornell Net Carbohydrate and Protein System as a tool to improve nitrogen utilization in commercial dairy herds. Prof. Anim. Sci. 28:370–378. https://doi.org/10.15232/S1080-7446(15)30370-3.
Hofherr, M. W. 2010. The effect of abomasal infusion of histidine and proline on milk composition and mammary amino acid utilization in high producing lactating dairy cows. MS thesis, Cornell University, Ithaca, NY.
Hogenboom, J. A., L. Pellegrino, A. Sandrucci, V. Rosi, and P. D'Incecco. 2019. Invited review: Hygienic quality, composition, and technological performance of raw milk obtained by robotic milking of cows. J. Dairy Sci. 102:7640–7654. https://doi.org/10.3168/jds.2018-16013.
Huhtanen, P., J. I. Nousiainen, H. Khalili, S. Jaakkola, and T. Heikkilä. 2003. Relationships between silage fermentation characteristics and milk production parameters: Analyses of literature data. Livest. Prod. Sci. 81:57–73. https://doi.org/10.1016/S0301-6226(02)00195-1.
Huhtanen, P., K. H. Südekum, J. Nousiainen, and K. J. Shingfield. 2010. Forage conservation, feeding value and milk quality. Pages 379–400 in Grassland in a Changing World. H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage, and A. Hopkins, ed. Universität Göttingen.
Innocente, N., and M. Biasutti. 2013. Automatic milking systems in the Protected Designation of Origin Montasio cheese production chain: Effects on milk and cheese quality. J. Dairy Sci. 96:740–751. https://doi.org/10.3168/jds.2012-5512.
Jacobs, J. A., and J. M. Siegford. 2012. Invited review: The impact of automatic milking systems on dairy cow management, behavior, health, and welfare. J. Dairy Sci. 95:2227–2247. https://doi.org/10.3168/jds.2011-4943.
Janštová, B., M. Dračková, K. Dlesková, Š. Cupáková, L. Necidová, P. Navrátilová, and L. Vorlová. 2011. Quality of raw milk from a farm with automatic milking system in the Czech Republic. Acta Vet. Brno 80:207–214. https://doi.org/10.2754/avb201180020207.
Johansen, M., P. Lund, and M. R. Weisbjerg. 2018. Feed intake and milk production in dairy cows fed different grass and legume species: A meta-analysis. Animal 12:66–75. https://doi.org/10.1017/S1751731117001215.
Johansson, M., Å. Lundh, R. De Vries, and K. S. Sjaunja. 2017. Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. J. Dairy Res. 84:154–158. https://doi.org/10.1017/S0022029917000140.
King, M. T. M., K. J. Sparkman, S. J. LeBlanc, and T. J. DeVries. 2018. Milk yield relative to supplement intake and rumination time differs by health status for fresh cows milked with automated systems. J. Dairy Sci. 101:10168–10176. https://doi.org/10.3168/jds.2018-14671.
Klungel, G. H., B. A. Slaghuis, and H. Hogeveen. 2000. The effect of the introduction of automatic milking systems on milk quality. J. Dairy Sci. 83:1998–2003. https://doi.org/10.3168/jds.S0022-0302(00)75077-6.
Kontopidis, G., C. Holt, and L. Sawyer. 2004. Invited review: β-Lactoglobulin: Binding properties, structure, and function. J. Dairy Sci. 87:785–796. https://doi.org/10.3168/jds.S0022-0302(04)73222-1.
Kung, L. Jr., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic com-

ponents of silages. J. Dairy Sci. 101:4020–4033. https://doi.org/10.3168/jds.2017-13909.
Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85:1165–1175. https://doi.org/10.3168/jds.S0022-0302(02)74179-9.
Manuelian, C. L., V. Vigolo, F. Righi, M. Simoni, S. Burbi, and M. De Marchi. 2021. MIR and Vis/NIR spectroscopy cannot authenticate organic bulk milk. Ital. J. Anim. Sci. 20:1810–1816. https://doi.org/10.1080/1828051X.2021.1954559.
Manzocchi, E., W. Hengartner, M. Kreuzer, and K. Giller. 2020. Effect of feeding hay vs. silages of various types to dairy cows on feed intake, milk composition and coagulation properties. J. Dairy Res. 87:334–340. https://doi.org/10.1017/S0022029920000801.
Manzocchi, E., B. Martin, C. Bord, I. Verdier-Metz, M. Bouchon, M. De Marchi, I. Constant, K. Giller, M. Kreuzer, J. Berard, M. Musci, and M. Coppa. 2021. Feeding cows with hay, silage, or fresh herbage on pasture or indoors affects sensory properties and chemical composition of milk and cheese. J. Dairy Sci. 104:5285–5302. https://doi.org/10.3168/jds.2020-19738.
Martz, F. A., A. T. Belo, M. F. Weiss, R. L. Belyea, and J. P. Goff. 1990. True absorption of calcium and phosphorus from alfalfa and corn silage when fed to lactating cows. J. Dairy Sci. 73:1288–1295. https://doi.org/10.3168/jds.S0022-0302(90)78794-2.
Mertens, D. R. 2009. Maximizing forage use by dairy cows take home messages. WCDS Adv. Dairy Tech. 21:303–319.
Mihaljev, Ž., S. Jaksic, and M. Ž. Baloš. 2015. Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products. J. Agroaliment. Processes Technol. 21:365–370.
Mollenhorst, H., M. M. Hidayat, J. van den Broek, F. Neijenhuis, and H. Hogeveen. 2011. The relationship between milking interval and somatic cell count in automatic milking systems. J. Dairy Sci. 94:4531–4537. https://doi.org/10.3168/jds.2011-4244.
Mordenti, A. L., N. Brogna, and A. Formigoni. 2017. Review: The link between feeding dairy cows and Parmigiano-Reggiano cheese production area. Prof. Anim. Sci. 33:520–529. https://doi.org/10.15232/pas.2016-01602.
Nielsen, N. I., T. Larsen, M. Bjerring, and K. L. Ingvartsen. 2005. Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. J. Dairy Sci. 88:3186–3200. https://doi.org/10.3168/jds.S0022-0302(05)73002-2.
Paulson, J., H. Jung, M. Raeth-Knight, and J. Linn. 2008. Grass vs. legume forages for dairy cattle. Pages 16–17 in Proceedings of the 69th Minnesota Nutrition Conference. Accessed Sep. 9, 2023. https://forages.org/Resources/Grass-vs-alfalfa-for-dairy-UM.pdf.
Penasa, M., V. Toffanin, N. Cologna, M. Cassandro, and M. De Marchi. 2016. Effects of dairy factory, milk casein content and titratable acidity on coagulation properties in Trentingrana dairy industry. J. Dairy Res. 83:242–248. https://doi.org/10.1017/S0022029916000212.
Priyashantha, H., Å. Lundh, A. Höjer, G. Bernes, D. Nilsson, M. Hetta, K. H. Saedén, A. H. Gustafsson, and M. Johansson. 2021. Composition and properties of bovine milk: A study from dairy farms in northern Sweden; Part II. Effect of monthly variation. J. Dairy Sci. 104:8595–8609. https://doi.org/10.3168/jds.2020-19651.
Raffrenato, E., D. A. Ross, and M. E. Van Amburgh. 2018. Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation. J. Dairy Sci. 101:9888–9900. https://doi.org/10.3168/jds.2018-15101.
Rasmussen, M. D., M. Bjerring, P. Justesen, and L. Jepsen. 2002. Milk quality on Danish farms with automatic milking systems. J. Dairy Sci. 85:2869–2878. https://doi.org/10.3168/jds.S0022-0302(02)74374-9.
Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in The Analysis of Dietary Fiber in Food. Marcel Dekker.
Rosmalia, A., I. G. Permana, and D. Despal. 2022. Synchronization of rumen degradable protein with non-fiber carbohydrate on microbial protein synthesis and dairy ration digestibility. Vet. World 15:252–261. https://doi.org/10.14202/vetworld.2022.252-261.
Salawu, M. B., A. T. Adesogan, and R. J. Dewhurst. 2002. Forage intake, meal patterns, and milk production of lactating dairy cows fed grass silage or pea-wheat bi-crop silages. J. Dairy Sci. 85:3035–3044. https://doi.org/10.3168/jds.S0022-0302(02)74389-0.
Schlegel, P., U. Wyss, Y. Arrigo, and H. D. Hess. 2018. Changes in macro-and micromineral concentrations in herbage during the harvesting and conservation processes. Grass Forage Sci. 73:918–925. https://doi.org/10.1111/gfs.12382.
Schmitt, S., S. Garrigues, and M. de la Guardia. 2014. Determination of the mineral composition of foods by infrared spectroscopy: A review of a green alternative. Crit. Rev. Anal. Chem. 44:186–197. https://doi.org/10.1080/10408347.2013.835695.
Serrapica, F., P. Uzun, F. Masucci, F. Napolitano, A. Braghieri, A. Genovese, R. Sacchi, R. Romano, C. M. A. Barone, and A. Di Francia. 2020. Hay or silage? How the forage preservation method changes the volatile compounds and sensory properties of Caciocavallo cheese. J. Dairy Sci. 103:1391–1403. https://doi.org/10.3168/jds.2019-17155.
Shingfield, K. J., P. Salo-Vaananen, E. Pahkala, V. Toivonen, S. Jaakkola, V. Piironen, and P. Huhtanen. 2005. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. J. Dairy Res. 72:349–361. https://doi.org/10.1017/S0022029905000919.
Simoni, M., A. Canepa, R. Pitino, A. Quarantelli, and F. Righi. 2021. Digestibility and sorting of hay-based total mixed rations employed in the Parmigiano-Reggiano area as affected by dietary particle size distribution. Large Anim. Rev. 27:123–132.
Sniffen, C. J., and A. Formigoni. 2006. How to control ruminal acidosis in the herd. Buiatria 38:79–89.
Spears, J. W. 1994. Minerals in forages. Pages 281–317 in Forage Quality, Evaluation, and Utilization. G. C. Fahey, Jr., ed. American Society of Agronomy Inc., Crop Science Society of America Inc., and Soil Science Society of America Inc. https://doi.org/10.2134/1994.foragequality.c7.
Steinshamn, H. 2010. Effect of forage legumes on feed intake, milk production and milk quality—A review. Anim. Sci. Pap. Rep. 28:195–206.
Stergiadis, S., C. Leifert, C. J. Seal, M. D. Eyre, J. H. Nielsen, M. K. Larsen, T. Slots, H. Steinshamn, and G. Butler. 2012. Effect of feeding intensity and milking system on nutritionally relevant milk components in dairy farming systems in the north east of England. J. Agric. Food Chem. 60:7270–7281. https://doi.org/10.1021/jf301053b.
Toušová, R., J. Ducháček, L. Stádník, M. Ptáček, and J. Beran. 2014. The comparison of milk production and quality in cows from conventional and automatic milking systems. J. Cent. Eur. Agric. 15:100–114. https://doi.org/10.5513/JCEA01/15.4.1515.
Tse, C., H. W. Barkema, T. J. DeVries, J. Rushen, and E. A. Pajor. 2018. Impact of automatic milking systems on dairy cattle producers' reports of milking labour management, milk production and milk quality. Animal 12:2649–2656. https://doi.org/10.1017/S1751731118000654.
van den Oever, S. P., A. Haselmann, M. Schreiner, B. Fuerst-Waltl, Q. Zebeli, H. K. Mayer, and W. Knaus. 2021. Hay versus silage: Does hay feeding positively affect milk composition? Int. Dairy J. 118:105024. https://doi.org/10.1016/j.idairyj.2021.105024.
Varlamova, E. G., and O. G. Zaripov. 2020. Beta-lactoglobulin—Nutrition allergen and nanotransporter of different nature ligands therapy with therapeutic action. Res. Vet. Sci. 133:17–25. https://doi.org/10.1016/j.rvsc.2020.08.014.
Vigolo, V., G. Niero, M. Penasa, and M. De Marchi. 2022. Effects of preservative, storage time, and temperature of analysis on detailed milk protein composition determined by reversed-phase high-performance liquid chromatography. J. Dairy Sci. 105:7917–7925. https://doi.org/10.3168/jds.2022-22069.
Wagner-Storch, A. M., and R. W. Palmer. 2003. Feeding behavior, milking behavior, and milk yields of cows milked in a parlor versus an automatic milking system. J. Dairy Sci. 86:1494–1502. https://doi.org/10.3168/jds.S0022-0302(03)73735-7.

Wiggans, G. R., and G. E. Shook. 1987. A lactation measure of somatic cell count. J. Dairy Sci. 70:2666–2672. https://doi.org/10.3168/jds.S0022-0302(87)80337-5.

Wiking, L., J. H. Nielsen, A. K. Båvius, A. Edvardsson, and K. Svennersten-Sjaunja. 2006. Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. J. Dairy Sci. 89:1004–1009. https://doi.org/10.3168/jds.S0022-0302(06)72166-X.

Wilkinson, J. M., and M. Rinne. 2018. Highlights of progress in silage conservation and future perspectives. Grass Forage Sci. 73:40–52. https://doi.org/10.1111/gfs.12327.

ORCIDS

Marica Simoni https://orcid.org/0000-0003-3920-9744
Rokia Temmar https://orcid.org/0000-0001-9213-0176
Massimo De Marchi https://orcid.org/0000-0001-7814-2525
Andrea Revello-Chion https://orcid.org/0000-0003-4207-895X
Marta Pozza https://orcid.org/0009-0001-0850-4022
Federico Righi https://orcid.org/0000-0001-9274-4143
Carmen L. Manuelian https://orcid.org/0000-0002-0090-0362