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VARIATION IN CHEESE MAKING PROPERTIES OF MILK FROM DAIRY COWS: FOCUS ON ANIMAL STATUS, FARMING SYSTEM AND CROSSBREEDING

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“Research is creating new knowledge”

- *Neil Armstrong*

Dedication

This dissertation is dedicated to my parents (Surja Kumar Saha and Arati Saha)

For their endless love, support and inspiration

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SUMMARY

Approximate 80% of world milk production comes from cows and nearly 40% of the total cow milk is processed into cheese. Thus, cheese making properties of cow's milk have received great interest from global cheese industry. In last decades, many scientific researches have investigated the factors able to affect the cheese making properties of milk. Among these, particular attention has been given to composition, handling, storage and pretreatment of milk, such as standardization and pasteurization, and to equipment and technology used to process milk into cheese. Also breeds of cows and specific characteristics of the animals, such as parity, days in milk and herds, have been investigated by several authors. Conversely, less is known about the effects of animal metabolic status on cheese-making properties of milk. Similarly, the relationships between the cheese-making properties of milk with different management strategies of dairy cows, such as specific farming systems, or the use of crossbreeding schemes need to be further investigated. Therefore, the present PhD thesis aimed to investigate the effects of rumen acidity status, that of summer transhumance dairying system and the implications of adopting specific schemes of crossbreeding on milk coagulation properties and cheese-making traits of dairy cows. In the present thesis, cheese-related traits of milk from individual cows were assessed by focusing on rumen acidity status (chapter 1), summer transhumance farming system (chapter 2) and crossbreeding (chapter 3 and chapter 4) effects on the milk composition, coagulation properties (single point milk coagulation traits and curd firming equation parameters), and cheese yield and nutrient recoveries in the curd. In addition, the quality (chemical components, rheological traits and sensory attributes) of cheese made from the milk of crossbred cows and purebred Holstein was also assessed (chapter 4).

More precisely, the goal of the first chapter was to investigate whether the rumen acidity status affects rumination time (**RT**), and the yield, composition and coagulation properties of milk (**MCP**), considering also cheese yield (**CY**) and milk nutrient recoveries in curd (**REC**). The increase in milk yield achieved in recent decades by the dairy sector has been sustained by feeding dairy cows with more concentrates and less forage. The use of high grain rations in dairy cows is related to an increase in rumen acidity. For this study, one hundred early-lactating Holstein cows with no clinical signs of disease and fed total mixed rations were used. Rumen fluid was collected once from each cow by rumenocentesis to determine pH and volatile fatty acid (**VFA**) content. The cows were classified according to the quartile of rumen acidity (**QRA**), a factor defined by multivariate analysis and associated with VFA and pH. Rumen fluid pH averaged 5.61 in the first quartile and 6.42 in the fourth, and total VFA content increased linearly with increasing rumen acidity. In addition, RT increased as rumen acidity increased, but only in the daily time interval from 08:00 to 12:00. Milk yield linearly decreased as rumen acidity increased, whereas QRA did not affect pH, fat or the protein content of milk. Furthermore, the MCP, assessed by lactodynamograph, and CY were unaffected by QRA. It is suggested that differences in rumen acidity have little influence on the nutrient content, coagulation properties and CY of milk.

The second chapter aimed to investigate the effects of summer transhumance of dairy cows to alpine pastures on body condition, milk yield and composition, coagulation properties, cheese yield and nutrient recoveries in curd. Summer transhumances of dairy cows to mountain pasture are widespread dairy farming system practiced in many European countries. In this study, total 12 multiparous Brown Swiss cows from lowland permanent farm (**PF**) were divided in two equal groups where one group remained at the PF and the other group was transferred to the alpine mountain pastures (**ALP**; 1860 m above sea level) from July to September. From June to October (once in a month), daily milk yield and body condition score (**BCS**) were recorded, and individual milk samples (n = 60, 2000 mL each) were collected for assessing milk composition, MCP, CY and REC traits. Compared with PF, ALP cows had a reduced milk yield and BCS, which was maintained on return to the PF, with greater fat and lower protein contents of milk. MCP, CY and REC traits were same for both groups of animals. Summer transhumance did not alter the cheese making efficiency of milk but depressed milk yield and consequently daily cheese yield, which was nearly 2 kg/d, lower for the ALP than the PF cows and was only partially recovered after returning to the PF in autumn.

The objective of the third chapter was to investigate the effects of long term systematic application of rotational crossbreeding on milk technological properties and cheese yield. Crossbreeding is a strategy to counter the declining fertility, resilience and longevity of purebred Holstein (**Ho**) cows. However, little is known of the effects of long-term systematic rotational crossbreeding on milk technological properties and cheese yield. In this study we compared the milk composition, coagulation properties and individual cheese yields of 468 purebred Ho and 648 crossbred (**CR**) cows obtained from two 3-breed rotational crossbreeding systems using Viking Red (**VR**), Montbéliarde (**Mo**) and Ho sires over 4 generations. Individual milk samples were collected once from 1116 primiparous and multiparous cows kept in two dairy herds, raised for the production of Grana Padano (high milk yield, total mixed ration based on corn silage) and Parmigiano Reggiano cheese (moderate milk yield, only dry feeds). In both herds, a 3-breed rotational mating system was used, in which Ho cows were first inseminated with VR, whereas Mo and Ho semen was used in the subsequent generations. In one herd, the sequence Mo-VR-Ho was also used. Individual milk samples were analyzed for milk composition, single-point MCP, and parameters for modeling curd-firming over time, whereas CY and REC traits were assessed through a laboratory cheese-making procedure. Compared with Ho, CR cows produced 5.8% less milk, with comparable fat but greater protein and casein contents, and lower lactose contents and somatic cell scores (**SCS**). Milk from CR cows reached a curd firmness of 20 mm more quickly, and exhibited greater curd firmness at 30, 45 and 60 min from rennet addition. Ho and CR cows yielded milk with very similar CY and REC traits. The milk fat content, SCS, curd firmness traits and CY of CR cows relative to the Ho cows differed in the two herds, and the favorable effects on the CR cows were more evident in the herd with the greatest milk yield and the worst MCP traits. Crossbred cows of the four generations performed similarly, with the exception of the better MCP of the milk from F1 CR cows. The two rotational systems using different sire-breed sequences also performed similarly. In summary, both rotational crossbreeding programs exhibited some advantage over the Ho purebred breeding system in terms of milk composition and MCP, but not CY. Future research is needed to investigate the interactions between crossbreeding schemes and dairy systems.

The fourth chapter focuses mainly on rheological, chemical and sensory properties of cheese made from milk obtained by purebred Ho and 3-breed rotational CR cows. Four generations of CR cows (F1 to F4) obtained using semen from VR, Mo and Ho sires were considered. A total of 120 individual milk samples (Ho: 40; CR: 80, 20 for each generation) were collected from evening milking in 6 different sessions of 20 cows each. Samples consisted on 2000 mL of raw full-fat milk, and they were refrigerated after collection and processed the subsequent day for assessing milk composition, casein micelle size, fat globule size, milk coagulation properties, fresh cheese yield and milk nutrient recoveries in the curd and model cheese preparation. The casein micelle and fat globule size in milk were measured by laser light scattering using Mastersizer 2000. The prepared cheeses, weighing 157.6 ± 23.2 g after 70 d of ripening, were evaluated for ripening loss, chemical, rheological and sensory attributes. Rheological properties include the instrumental color assessment, through a Minolta colorimeter, and the texture analysis, through a TA.XTplus texture analyzer. Sensory analysis was performed by 6 panelists, which assessed cheese samples (score: low, 1 to high, 10) for five main sensory attributes related to appearance (color), smell intensity, flavor intensity, taste attributes (sweet, salt, sour, bitter and umami), and texture characteristics (elasticity, firmness and moisture). Results show that, compared with the milk from purebred Ho cows that from CR cows had greater contents of protein and casein and lower contents of lactose and somatic cell score. Moreover, milk from CR cows had smaller average casein micelle size compared to milk from Ho cows, with comparable, although tendentially smaller, fat globule size (d_{43}, d_{32}). Also, milk from CR cows showed shorter time to reach a curd firmness of 20 mm and greater curd firmness at 45 and 60 min after rennet addition. Milk from CR and Ho cows exhibited similar cheese yield and milk nutrient recovery traits. In addition, the weight, chemical composition, rheological (color, hardness, cohesiveness, elasticity index and chewiness) and sensory attributes of individual model cheeses were comparable for Ho and CR cows. Among crossbred cows, VR sired crossbred cows displayed a positive effect on milk composition (protein and casein), curd firming properties, CY and REC traits and weight of cheese for fresh and ripened condition, whereas Mo sired crossbred cows had opposite trends for these traits. This study indicates that 3 breed rotational crossbreeding scheme did not exert any negative effect in cheese making properties and cheese attributes and seems to adapt to farming systems particularly focused toward cheese production.

The knowledge of this research provides new insights in factors affecting cheese making properties which have significant importance for farmer and cheese industry point of view.

RIASSUNTO

Circa l'80% della produzione mondiale di latte proviene dalle vacche e quasi il 40% del latte vaccino viene trasformato in formaggio. Pertanto, le proprietà di coaugulazione del latte vaccino hanno suscitato grande interesse da parte dell'industria casearia globale. Negli ultimi decenni, molte ricerche scientifiche hanno studiato i fattori in grado di influenzare le proprietà casearie del latte. Tra questi, particolare attenzione è stata data alla composizione, manipolazione, conservazione e pre-trattamento del latte, come la standardizzazione e la pastorizzazione, le attrezzature e le tecnologie utilizzate per la trasformazione del latte in formaggio. Anche la razza e le caratteristiche specifiche degli animali, come l'ordine di parto, i giorni di lattazione e l'allevamento, sono state studiate da diversi autori. Al contrario, poche informazioni si hanno sugli effetti dello stato metabolico delle vacche sulle proprietà coaugulative del latte. Analogamente, devono essere ulteriormente studiate anche le correlazioni tra le proprietà casearie del latte con le diverse strategie di gestione delle vacche, relative al sistema di allevamento, o all'uso di schemi di incrocio. Pertanto, la presente tesi di dottorato mira a studiare gli effetti dell'acidità ruminale, dell'alpeggio nella stagione estiva e le implicazioni dell'adozione di schemi specifici di incroci sulle proprietà della coagulazione del latte e sui caratteri di produzione del formaggio dal latte vaccino. Nella presente tesi, i caratteri relativi alla produzione di formaggio sono stati ottenuti da campioni individuali, concentrandosi sullo stato dell'acidità del rumine (capitolo 1), sull'effetto dell'alpeggio nella stagione estiva (capitolo 2) e sull'adozione di uno schema di incrocio (capitolo 3 e capitolo 4) sulla composizione del latte, le proprietà di coagulazione, la resa in formaggio e il recupero dei nutrienti nella cagliata. Inoltre, è stata valutata anche la qualità, in termini di composizione

chimica e catteristiche reologiche e sensoriali, del formaggio prodotto dal latte di vacche di razza razza pura Holstein o meticce (capitolo 4).

Più precisamente, l'obiettivo del primo capitolo è stato quello di studiare le relazioni tra lo stato di acidità del rumine ed il tempo di ruminazione (**RT**), la composizione e le proprietà di coagulazione del latte (**MCP**), considerando anche la resa del formaggio (**CY**) e i recuperi di nutrienti del latte in cagliata (**REC**). L'aumento della resa del latte raggiunto negli ultimi decenni dal settore lattiero-caseario è stato sostenuto e dovutodal fatto che le vacche da latte sono alimentate con maggiori quantità di concentrati e meno foraggi. L'uso di razioni con elevate quantità di concentrato nelle vacche da latte è correlato ad un aumento dell'acidità del rumine. In questo studio sono state utilizzate cento vacche di razza Frisona che si trovavano nella prima fase di lattazione e che non avessero segni clinici di malattia, alimentate con razioni di tipo unifeed. Da ciascuna vacca è stato prelevato un campione di liquido ruminale mediante rumenocentesi sul quale è stato misurato il pH ed effettuata l’analisi degli acidi grassi volatili (**AGV**). Le bovine sono state classificate in base al quartile di acidità del rumine (**QRA**), un fattore definito dall'analisi multivariata e associato agli AGV e al pH. Il pH del liquido ruminale è risultato in media di 5,61 nel primo quartile e di 6,42 nel quarto, e il contenuto totale di AGV è aumentato linearmente con l'aumentare dell'acidità del rumine. Inoltre, la RT è aumentata all'aumentare dell'acidità del rumine, ma solo nell'intervallo di tempo giornaliero dalle 08:00 alle 12:00. La resa del latte è diminuita linearmente all'aumentare dell'acidità del rumine, mentre il QRA non ha influenzato il pH, il grasso o il contenuto proteico del latte. Inoltre, il MCP, valutato dal lattodinamografo, e il CY, non sono stati influenzati dal QRA. I risultati ottenuti suggeriscono che le differenze di acidità del rumine non abbiano influenza sul contenuto nutrizionale, sulle proprietà della coagulazione e sul CY del latte.

Il secondo capitolo mirava a studiare gli effetti della transumanza estiva delle vacche da latte sui pascoli alpini sulla condizione corporea, la composizione e la resa del latte, le proprietà di coagulazione, la resa di formaggio e recupero di nutrienti nella cagliata. L'alpeggio delle vacche da latte nei pascoli di montagna è un sistema di allevamento diffuso in molti paesi europei. In questo studio, 12 vacche pluripare di razza Bruna Italiana provenienti da allevamenti permanenti di pianura (**PF**) sono state divise in due gruppi omogenei, per cui un gruppo è rimasto al PF e l'altro gruppo è stato trasferito in alpeggio (**ALP**; 1860 m sul livello del mare) da luglio a settembre. Nel periodo da giugno a ottobre (una volta al mese), per ciascuna bovina sono stati raccolti i dati della produzione giornaliera del latte e il punteggio della condizione corporea (BCS) e sono stati effettuati campionamenti individuali di latte (n = 60, 2000 mL ciascuno) per valutare la composizione, i caratteri MCP, CY e REC. Le vacche ALP hanno evidenziato una produzione di latte e una condizione corporea (**BCS**) inferiore rispetto al gruppo PF. Inoltre il latte delle ALP aveva un maggior contenuto di grassi e un minor contenuto di proteina rispetto al gruppo delle bovine PF. I tratti MCP, CY e REC sono risultati simili per entrambi i gruppi di animali. L'alpeggio nella stagione estiva non modifica la capacità del latte ad essere trasformato in formaggio, ma riduce la produzione del latte e di conseguenza la produzione giornaliera di formaggio, che era quasi 2 kg/d più bassa per le ALP rispetto alle vacche PF e veniva recuperata solo parzialmente dopo essere tornata alla PF in autunno.

L'obiettivo del terzo capitolo era di studiare gli effetti dell'applicazione sistematica a lungo termine di uno schema di incrocio a rotazione a tre vie sulle proprietà tecnologiche del latte e la resa in formaggio. L'incrocio è una strategia per ridurre il calo della fertilità, la capacità di recupero e la longevità delle vacche di razza Frisona (**Ho**) allevate in purezza. Tuttavia, si sa poco degli effetti dell'incrocio sistematico a lungo termine sulle proprietà tecnologiche del latte e sulla resa in formaggio. In questo studio abbiamo confrontato la composizione e le proprietà di coagulazione del latte e le rese individuali in formaggio di 468 vacche di razza pura Ho e 648 vacche meticce (**CR**) ottenute da due sistemi di incroci rotazionali basati sull'uso di 3 razze, Viking Red (**VR**), Montbéliarde (**Mo**) e Ho considerando 4 generazioni successive di incrocio. Campioni individuali di latte sono stati raccolti da 1116 bovine primipare e pluripare allevate in due aziende. Le aziende considerate destinavano il latte alla produzione di Grana Padano (azienda A, alta produzione di latte, alimentate con unifeed a base di insilato di mais) e a quella di Parmigiano Reggiano (azienda B, moderata produzione di latte, alimentazione a secco). In entrambe le aziende è stato utilizzato un sistema di accoppiamento a rotazione a 3 vie, in cui le vacche Ho sono state inseminate per la prima volta con la VR, mentre il seme Mo e Ho è stato utilizzato nelle generazioni successive sulle vacche F2 ed F3, rispettivamente. In una azienda, è stata utilizzata anche la sequenza Mo-VR-Ho. I singoli campioni di latte sono stati analizzati per la composizione, MCP a punto singolo e parametri per la modellizzazione nel tempo del rassodamento della cagliata, mentre il recupero di CY e REC sono stati valutati attraverso una procedura di produzione di formaggio in laboratorio. Rispetto a Ho, le vacche CR hanno prodotto il 5,8% in meno di latte, con livello di grasso paragonabile ma maggiore contenuto di proteine e caseina, contenuto di lattosio e punteggi delle cellule somatiche inferiori. Il latte delle vacche CR ha raggiunto una consistenza della cagliata di 20 mm più rapidamente e ha mostrato una maggiore compattezza della cagliata a 30, 45 e 60 minuti dall'aggiunta del caglio. Le vacche Ho e CR hanno prodotto latte con caratteristiche CY e REC molto simili. Il contenuto di grassi del latte, SCS, caratteri di consistenza della cagliata e CY delle vacche CR rispetto alle vacche Ho differivano nelle due mandrie e gli effetti favorevoli sulle vacche CR erano più evidenti nell'azienda con maggior produzione di latte e peggiori caratteristiche MCP. Le vacche meticce di diversa generazione si sono comportate in modo simile, ad eccezione del migliore MCP del latte delle vacche F1. Anche i due sistemi di rotazione che utilizzavano sequenze di razza differenti hanno prodotto risultati simili. In sintesi, entrambi i programmi di incrocio a rotazione hanno mostrato alcuni vantaggi rispetto al sistema di allevamento basato sulla Ho pura in termini di composizione del latte e MCP, ma non di CY. Sono necessarie ricerche future per studiare le interazioni tra schemi di incroci e sistemi lattiero-caseari.

Il quarto capitolo si concentra principalmente sulle proprietà reologiche, chimiche e sensoriali del formaggio prodotto dal latte ottenuto da vacche di razza Ho e meticce prodotte nell'ambito di uno schema di incrocio a rotazione a 3 vie CR. Sono state prese in considerazione quattro generazioni di vacche CR (da F1 a F4) ottenute utilizzando seme di VR, Mo, e Ho. Un totale di 120 campioni individuali di latte (Ho: 40; CR: 80, 20 per ogni generazione) sono stati raccolti dalla mungitura serale in 6 diversi campionamenti di 20 vacche ciascuna. I campioni consistevano in 2000 mL di latte intero crudo, e sono stati refrigerati dopo la raccolta ed analizzati il giorno successivo per valutare la composizione del latte, la dimensione delle micelle della caseina, la dimensione del globulo di grasso, le proprietà della coagulazione del latte, la resa del formaggio fresco e recupero di nutrienti nel latte nella cagliata e preparazione del modello di formaggio. Le dimensioni delle micelle di caseina e dei globuli di grasso sono state misurate utilizzando un Mastersizer 2000. I formaggi preparati, che pesavano 158 ± 23 g dopo 70 d di maturazione, sono stati valutati per perdita di maturazione, caratteristiche chimiche, reologiche e sensoriali. Le proprietà reologiche comprendono la valutazione strumentale del colore, attraverso un colorimetro Minolta, e l'analisi della tenerezza, attraverso un lo strumento TA.XTplus. L'analisi sensoriale è stata eseguita da 6 panelisti, che hanno valutato i campioni di formaggio (punteggio: basso, da 1 a alto, 10) per cinque principali attributi sensoriali relativi all'aspetto (colore), intensità dell'odore, intensità del sapore, attributi del gusto (dolce, salato, acido, amaro e umami) e caratteristiche di consistenza (elasticità, consistenza e umidità). I risultati mostrano che, rispetto al latte delle vacche di razza Ho, quello delle vacche CR presentava un maggiore contenuto di proteine e caseina e un contenuto inferiore di lattosio e punteggio delle cellule somatiche. Inoltre, il latte delle vacche CR presentava una dimensione media delle micelle di caseina inferiore rispetto al latte delle vacche Ho, con dimensioni di globuli di grasso tendenzialmente più piccole. Inoltre, il latte delle vacche CR ha mostrato un tempo più breve per raggiungere una compattezza della cagliata di 20 mm e una maggiore compattezza della cagliata 45 e 60 minuti dopo l'aggiunta del caglio. Il latte delle vacche CR e Ho presentava una resa simile del formaggio e i caratteri di recupero dei nutrienti del latte. Inoltre, peso, composizione chimica, reologica (colore, durezza, coesione, indice di elasticità e gommosità) e attributi sensoriali dei singoli formaggi erano comparabili per le vacche Ho e CR. Tra le bovine meticce, quelle prodotte da padre VR hanno mostrato un effetto positivo sulla composizione del latte (proteine e caseina), proprietà rassodanti della cagliata, tratti CY e REC e peso del formaggio per condizioni fresche e mature, mentre quelle prodotte da padre Mo hanno avuto tendenze opposte per queste caratteristiche. Questo studio indica che il sistema di incrocio a rotazione a 3 vie non ha esercitato alcun effetto negativo sulle proprietà di produzione del formaggio e sulle caratteristiche del formaggio e sembra adattarsi ai sistemi di allevamento particolarmente incentrati sulla produzione di formaggio.

La conoscenza di questa ricerca fornisce nuove informazioni sui fattori che influenzano le proprietà di produzione del formaggio che hanno un'importanza significativa dal punto di vista degli allevatori e dell'industria lattiero-casearia.

GENERAL INTRODUCTION

Cheese is a delicious and nutritious food that plays a very important role in the diet of human. About 36% of total cow milk is used for cheese production (International Dairy Federation, 2016) and this rate has increased by 23%, during the last decade (Sanchez et al., 2018). The European Union (EU-27) is the top producer of cheese in the world, approximately 50% of the world production (US Department of Agriculture; Economic Research Service, 2018). Average annual growth rate of cheese production was 1.56% from 2013 to 2018 in the European Union (CLAL, 2019). Among European countries, Italy is the third largest cheese producer country (Figure 1) where 75% milk is destined for cheese production and 50% of the total milk is processed for PDO (Protected Designation of Origin) cheeses (Benedet et al., 2018).

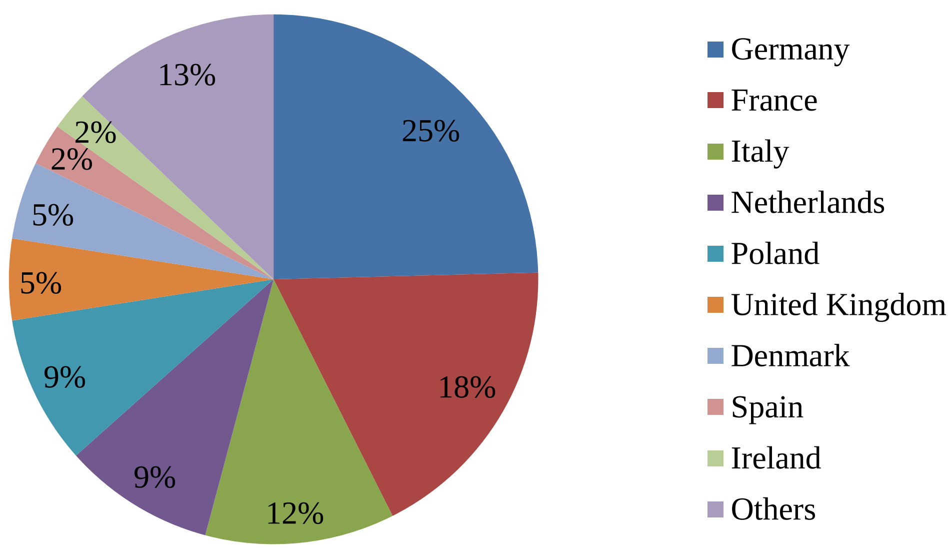

Figure 1. Production of cheese from cows' milk (January – December, 2018).

(Source: CLAL, 2019)

In this situation, cheese making properties are important for milk processing industry. On the basis of this consideration, some specific steps related to cheese making properties will be introduced here.

Milk Coagulation Properties

The milk coagulation properties (**MCP**) are important steps to determine the cheese making properties of milk (Wedholm, 2006), as MCP traits can influence the cheese yield and cheese quality (Stocco et al., 2017). Several techniques (Klandar et al., 2007; Troch et al., 2017) can be used to assess MCP but the most common method use in both research and industry level is lactodynamogarphy (Stocco et al., 2017). This is a mechanical device evaluates the clotting aptitude of milk (Caroli et al., 1990) and has been widely used for some decades (Annibaldi et al., 1977; McMahon and Brown, 1982; Bittante et al., 2011). Traditionally, three single point MCP traits are recorded by using lactodynamography, that records rennet coagulation time (RCT), curd firmness over 30 minutes time after rennet addition (a_{30}, mm) and time to reach curd firmness of 20 mm (k_{20}, min) (Bittante, 2011).

This technique monitors the viscosity of milk samples after addition of rennet (enzyme) at fixed temperature during a particular period of time and allows us to evaluate several milk samples at the same time. During lactodynamographic analysis, milk temperature is kept constant (35^{o}C) and the device measures the tiny forces that act on the pendulum during coagulation (gelation) when they oscillated in a linear manner; the greater the extent of coagulation, the lesser the pendulum swing (Bittante et al., 2012). The detected signals are recorded and produce a typical Formagraph (bell shape) output, as shown in Figure 2.

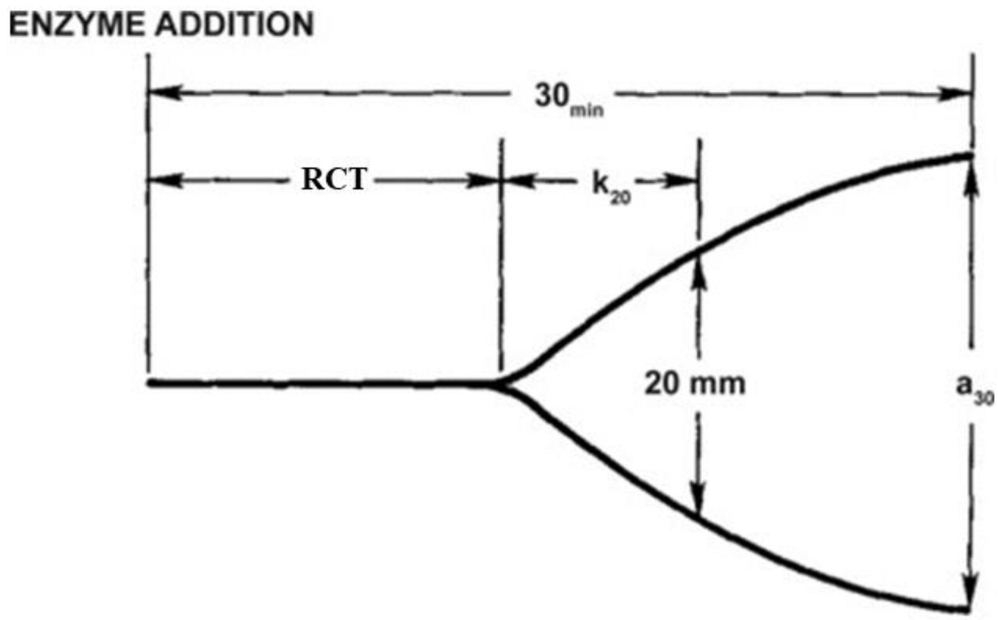

Figure 2. Lactodynamographic curve as a recorded with the original Formagraph.
(Source: Bittante et al., 2011)

The major problems related to assess the traditional MCP are incidence of non-coagulating samples that do not coagulate within 30 min of rennet addition, late coagulating samples failing to acquire curd firmness of 20 mm (k_{20}) and curd firmness after 30 min (a_{30}). These limitations are overcome by prolonging the observation period and by using curd firming modeling (CFt) over time using data measured by lactodynamography (Bittante et al., 2011, 2013). During the lactodynamographic test, all 240 curd firmness point recorded from each individual milk sample (1 every 15 s for 60 min observation) were saved to be modeled by using following equation:

$$CF_t = CF_p \times \left(1 - e^{-k_{CF} \times (t - RCT_{eq})}\right) \times e^{-k_{SR} \times (t - RCT_{eq})}$$

Where, new parameters related to milk coagulation properties are explored by curd firming modeling (CFt) equation and allow the estimation of RCT (RCT_{eq}), the potential asymptotic curd firmness (CF_p), maximum curd firmness (CF_{max}), time to reach maximum curd firmness (t_{max}), the rate of curd firming (k_{CF}) and syneresis rate (k_{SR}). The CFt model is illustrated in Figure 3.

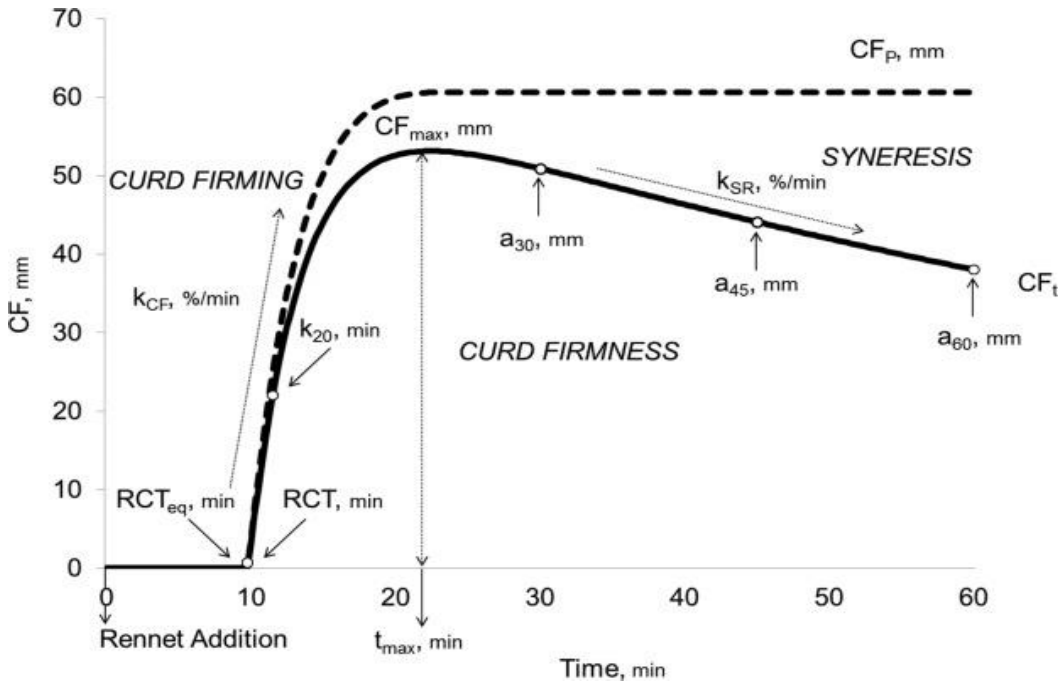

Figure 3. Modeling prolonged observations of curd firmness at time t model parameters. The solid black line represents the modeling of frequent observations of curd firmness (CF, one observation every 15 s) at time t (CFt). The model parameters are RCT_{eq} = rennet coagulation time from enzyme addition (min) estimated using the CF_t equation; CF_P = potential asymptotical curd firmness (mm); k_{CF} = curd-firming rate constant (% × min^{-1}); k_{SR} = syneresis rate constant (% × min^{-1}). Two maximum CF traits are calculated from the model equation: CF_{max} = maximum curd firmness (mm); and t_{max} = time to CF_{max} (min). The open circles represent traditional single point coagulation properties. RCT = the time from enzyme addition to the last point below 1 min, min; k_{20} = the difference between the time to the first point greater than 20 mm CF and RCT, min; and a_{30}, a_{45}, a_{60} = the CF value recorded 30, 45, and 60 min after enzyme addition, mm.

(Source: Bittante et al., 2017)

Cheese Yield

In general, cheese making is a dehydration process where fat and protein contents of milk are concentrated and resulting cheese yield (Emmons, 1993). Cheese yield (**CY**) is an important determinant for the efficiency and profitability of dairy industry (Emmons, 1993). It is used to determine milk payment, asses the effectiveness of processing modifications and appraise effectiveness of possible new ingredients for use in cheese manufacture (Banks, 2007).

Cheese yield can be defined in several ways. The basic definition of CY is the weight of cheese in kg produced from 100 kg of milk, or the volume of milk in liters required to produce one ton of cheese (Banks, 2007). In brief, Fenelon and Guinee, (1999), defined CY the quantity of cheese obtained from a given amount of milk. The calculation of actual CY requires the measurement of the weight of all inputs (milk, starter and salt) and outputs (cheese and whey) during cheese making process. Efficiency of cheese making process relies on the recovery of milk components in the curd, and their loss in the whey (Banks, 2007). Most of the predictive formulas for estimating CY based on the weight and chemical compositions (fat, protein and total solids) of the milk and whey (Verdier-Metz et al., 2001; Cipolat-Gotet et al., 2013; Stocco et al., 2018).

Cheese making experiments are expensive, time consuming, labor intensive and allow only for small replicates when performed in cheese making plant. Also, most of the studies involving cheese making procedures have used bulk milk (Vacca et al., 2018). It is well known that individual CY is important to predict the daily cheese yield (Cipolat-Gotet et al., 2013) which has economic importance for dairy farmers. Moreover, individual CY has importance to evaluate and selection of individual animals on their cheese yield ability. For this reason, several studies (Hurtaud et al., 1995; Wedholm et al., 2006; Cologna et al., 2009; Cipolat-Gotet et al., 2013, 2016) have been performed to develop individual laboratory cheese making procedures able to assess individual CY, using sample size ranging from 1 mL to around 10 L milk. Several steps are involved in individual laboratory cheese making operation: individual milk sampling; milk analysis; milk weighing and heating; possible starter culture preparation and inoculation;

pH measurement; rennet preparation and addition; gelation time recording and curd cutting; whey drainage, sampling and weighing; curd sampling and analysis; wheel formation, compression, salting, weighing and model cheese ripening and analysis. Although several individual laboratory cheese making procedures developed, the laboratory micro-cheese making procedure proposed by Stocco et al. (2018) showed good repeatability and reproducibility and requires 1.5 L milk per sample. The detail procedure is summarized in flow chart (see appendix).

Recent modification of the cheese making assessment proposed by Cipolat-Gotet et al. (2016) is 9-MilCA (see appendix), using 9 mL of milk to measure individual CY. The 9-MilCA method is efficient because of large number of milk samples can be analyzed per session/day (Vacca et al., 2019). It also gives information from rennet-to-milk addition to separation of curd from whey in only single analysis.

Both proposed laboratory cheese making techniques stimulate the industrial cheese making process. Compared to industrial cheese making process, these laboratory cheese making procedures have some advantages such as need small quantities of milk; reduce time and cost for experiments; more possible treatments or replicates per day and the ability to measure cheese yield from individual animal. Among these two laboratory techniques, micro cheese making procedure produced a small cheese which is possible to ripen for further explore other characteristics of cheese (chemical, rheological and sensory traits), but difficult to analyze large number of samples per session/day. Conversely, large number of milk samples can be analyzed per session/day by using 9-MilCA method but difficult to ripened this produced cheese to further explore of other characteristics of cheese because of use only 9- mL of milk in this procedure.

Characteristics of Cheese

Overall, cheese consumption has increased in worldwide and simultaneously consumers have increasingly been demanding consistent physiochemical properties, sensory and nutritional quality of cheese at a reasonable cost (Lamichhane et al., 2018). Thus, dairy industry has to monitor the outcomes of the entire process of cheese making to produce cheese with good characteristics. MCP and CY are important from a technological point of view, but need to consider consumers expectations for good quality products. Therefore, in a food-chain perspective also traits related to cheese quality should be taken into account. Among these, chemical, rheological and sensory traits are important that's can influence the cheese quality.

Chemical compositions (such as protein, fat, and or dry matter content) of cheese are important to the cheese makers and consumer because of consumer-driven demand for healthy foods. Rheological properties (color and texture) and sensory attributes have vital roles in determining cheese quality because of these parameters are primarily considered by consumers when they buy the cheese (Pinho et al., 2004; Eroglu et al., 2016). Thus, the research on rheological and sensory attributes on cheese has significant impacts for consumers and global cheese industries point of view. To determine rheological properties of a cheese based on color and texture, food industries relies on instrumental system of color and texture measurement (Pinho et al., 2004; Eroglu et al., 2016). These instrumental measurement stimulates the visual action of human (Pinho et al., 2004) for color and compression action of teeth during mastication for texture (Bourne, 1978). Instrumental measurement of color provides information about lightness, yellowness and redness whereas instrumental texture profile analysis delivers hardness, cohesiveness, elasticity index and chewiness. Like chemical and rheological properties of cheese, sensory properties are important attributes which represents the wholesome image of cheese from consumer point of view. The following sensory attributes related to appearance (color), smell intensity, flavor intensity, taste attributes (sweet, salt, sour, bitter and umami), and texture characteristics (elasticity, firmness and moisture) are important for consumer point of view. Both rheological and sensory attributes of cheese are influenced by the chemical composition of cheese and biochemical changes occurring during the ripening (Awad et al., 2006). For instance, fat content of cheese relate with taste, texture and appearance (Rudan et al., 1999), whereas protein content influence the texture and flavor attributes of cheese (Eroglu et al., 2015).

Sources of Variation of Cheese Making Properties

The identification of the sources of variation of cheese making properties is important as they can support farmers and cheese processors to manage efficiently their production goals. In last decades, several studies have investigated the factors able to affect the cheese making properties of milk. Among these, particular attention has been given to composition, handling, storage and pretreatment of milk, such as standardization and pasteurization, and to equipment and technology used to process milk into cheese (Banks et al., 2007). Also breeds of cows and specific characteristics of the animals, such as parity, days in milk and herds, have been investigated by several authors (Martin et al., 2009; Cipolat-Gotet et al., 2013; Stocco et al., 2017, 2018). Many factors can influence the cheese making properties, including milk composition, species, breed, lactation stage, parity, feeding, health and cheese making conditions (Banks et al., 2007). On the other hand, effects of animal metabolic status such as acidosis, ketosis and fatty liver etc. and specific farming system (summer transhumance) or the use of crossbreeding scheme on cheese making properties of milk have received less attention. Among different metabolic conditions in dairy cows, rumen acidity is commonly found in lactating dairy cows.

Rumen Acidity. Over the last few decades, the increase in milk yield achieved by the dairy sector has been largely sustained by concurrent increase the concentrate feeding to dairy cows (Plaizier et al., 2008). This may lead to increase the risk of rumen acidity, as an excess of grain induces rumen microorganisms to convert carbohydrates into volatile fatty acids at a rate exceeding rumen absorption, buffering and outflow capacity (Plaizier et a., 2008) by reducing the rumen fluid pH and possible increase of ruminal lactic acid concentration. Rumen acidity can alter the rumen microorganism compositions which can alter the rumen fermentation pattern (Pugh and Baird, 2012) leading to alteration of milk composition. The effects of rumen acidity on milk yield and composition are controversial, while those on milk coagulation properties and cheese yield have not yet been explored.

Farming System (Summer Transhumance). Summer transhumance of dairy cows is a seasonal pastoral system practiced in many European countries, especially in the Alps region from pre-historic times (Sturaro et al., 2013). This practice is important for farmers because of additional forage supply for mountain dairy farms and plays a role for preservation of landscape, biodiversity, and natural habitats and conservation of local traditional dairy products (Zendri et al., 2016), but it may affect cows' physiological and nutritional status which may turn in milk yield, composition and processing qualities. Several studies have evaluated the quality of milk and cheese during summer transhumance (Bovolenta et al., 1998, 2009; Leiber et al., 2006; Romanzin et al., 2013). Conversely, the knowledge on comparing cow's kept on lowland farms with those temporarily moved to highland pasture during the summer are not available.

Crossbreeding. Crossbreeding in dairy cows is growing towards interest because of declining fertility, health, and longevity of purebred dairy cows. Benefits of crossbreeding have been well documented for production, health, fertility, and survival of dairy cattle in several studies (López-Villalobos et al., 2000; Heins et al., 2012; Hazel et al., 2017a,b; Shonka-Martin et al., 2019). Conversely, little is known of the effects of long-term systematic rotational crossbreeding on milk technological properties, cheese yield and cheese characteristics.

These newly identified sources of variation allow a new insight into the cheese making properties of milk from dairy cows.

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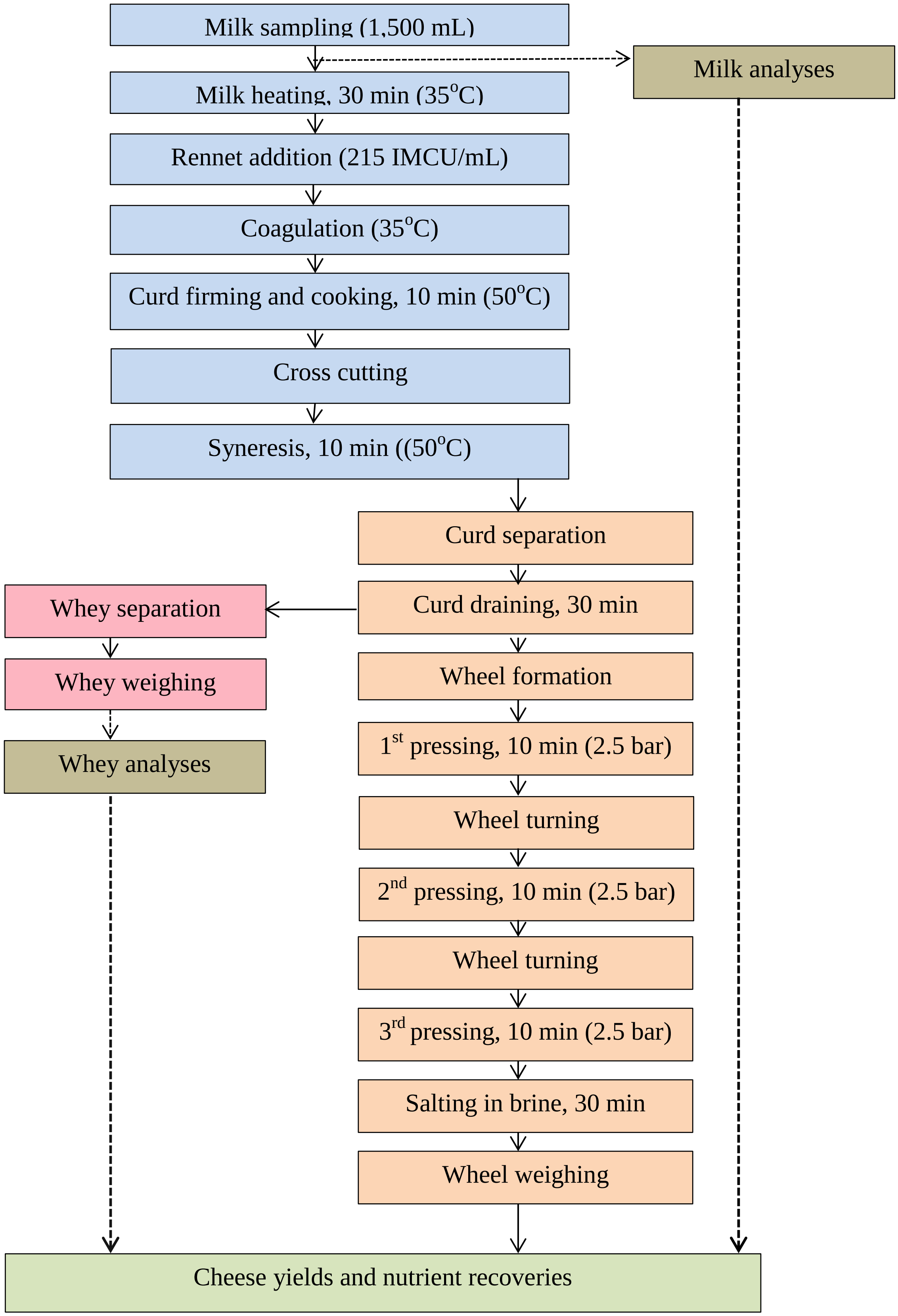
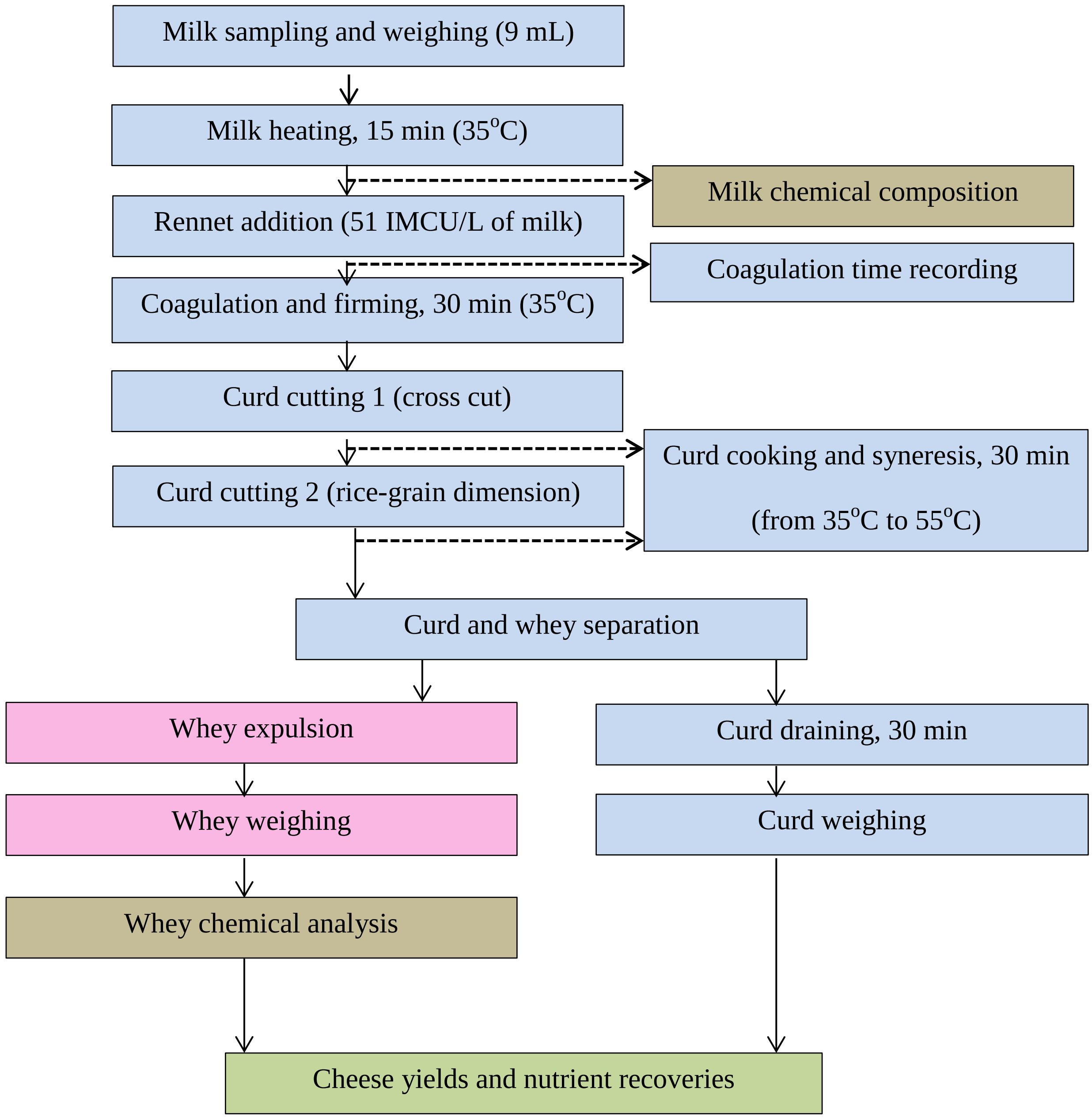
APPPENDIX

Flow chart for the laboratory micro-cheese making procedure (Source: Stocco et al., 2018)



APPENDIX

Flow chart for the 9-MilCA cheese making procedure (Source: Cipolat-Gotet et al., 2016)



AIMS OF THE THESIS

The main objective of this dissertation was to investigate the effects of some sources of variation of cheese making properties related to farming system, animal health and crossbreeding of dairy cows.

The specific objectives were:

- To explore whether ruminal acidity status of Holstein dairy cows would affect the production, composition, coagulation properties and cheese yield of milk.
- To investigate the effects of summer transhumance to alpine pastures of Brown Swiss cows on yield, composition, and coagulation properties of milk, and on individual cheese yield compared with a control group kept in lowland farm conditions.
- To compare purebred Holstein and crossbred cows from a 3-breed rotational crossbreeding scheme including Viking Red, Montbéliarde and Holstein breeds for milk production and composition, milk coagulation properties and cheese yield and milk nutrient recoveries in curd using a laboratory cheese making procedure based on 9-mL frozen milk samples.
- To compare the purebred Holstein and crossbred cows from a 3-breed rotational crossbreeding scheme including Viking Red, Montbéliarde and Holstein breeds for cheese yield, rheological, chemical and sensory properties of ripened model cheese, using a model-cheese procedure based on 1.5 L of fresh milk samples.

CHAPTER 1

A study on the effects of rumen acidity on rumination time and yield, composition, and technological properties of milk from early lactating Holstein cows

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ABSTRACT

The use of high grain rations in dairy cows is related to an increase of the acidity of the rumen. This study investigated whether rumen acidity status affects rumination time (**RT**), and the production, composition, coagulation properties (**MCP**) and cheese yield (**CY**) of milk. One-hundred early-lactating Holstein cows with no clinical signs of disease and fed total mixed rations were used. Rumen fluid was collected once from each cow by rumenocentesis to determine pH and volatile fatty acid (**VFA**) content. The cows were classified according to the quartile of rumen acidity (**QRA**), a factor defined by multivariate analysis and associated to VFA and pH. Rumen fluid pH averaged 5.61 in the first quartile and 6.42 in the fourth, and total VFA content increased linearly with increasing rumen acidity. Also RT increased with rumen acidity increase, but only in the daily time interval 08.00 to 12.00. Milk yield linearly decreased as rumen acidity increased, whereas QRA did not affect pH, fat and protein content of milk. Also MCP, assessed by lactodynamograph, and CY were unaffected by QRA. It is suggested that even huge differences in rumen acidity would have little influence on nutrient content, coagulation properties and CY of milk.

Keywords: dairy cows, rumen acidity, volatile fatty acids, rumination time, milk yield and composition, milk coagulation properties, cheese yield.

INTRODUCTION

The increase in milk yield achieved in recent decades by the dairy sector has been largely sustained by a concurrent increase in the use of concentrates in the rations for dairy cows (Plaizier et al., 2008). This may lead to increasing rumen acidity, as an excess of grain induces rumen microorganisms to convert carbohydrates into volatile fatty acids (**VFAs**) at a rate exceeding rumen absorption, buffering and outflow capacity (Plaizier et al., 2008), with a concomitant reduction of the rumen fluid pH and possible rise of ruminal lactic acid concentration.

Periods of moderate rumen pH depression are defined as subacute ruminal acidosis (**SARA**) (Krause and Oetzel, 2006). Field diagnosis of SARA is primarily based on the measurement of the ruminal pH (Humer et al., 2018), but diagnostic uncertainty arises from disagreements over the rumen pH threshold and the duration of periods of pH depression (Plaizier et al., 2006; Krause and Oetzel, 2006). A drop in ruminal pH to non-physiological levels seems widespread (Morgante et al., 2007; Kleen et al., 2013) and is regarded with particular concern by the dairy industry (DeVries et al., 2006). Depressed rumen pH has been linked to lower feed intake (Danscher et al., 2015) and milk yield (Stone, 1999) and an increased risk of liver abscesses and lameness (Plaizier et al., 2006). Rumination time (**RT**) could also be associated with depressed rumen pH, as it is related to saliva production, which can help to buffer ruminal fluid (Krause and Oetzel, 2006).

The overall acidity status of the rumen content is expressed not only by the pH, but also by the amount and concentration of different VFAs in the rumen fluid, which in turn are related to the saliva production and rumination activity. Rumen acidity can alter the rumen microorganism composition, which affects the ruminal fermentation pattern and the acetate to propionate ratio, leading to alterations of milk components, particularly the milk fat content (Lessire and Rollin, 2013). The association between ruminal pH, VFA concentration, and milk composition is controversial, as some authors found that rumen pH depression is related to a reduction in milk fat content (Danscher et al., 2015), others did not (Enjalbert et al., 2008; Kleen et al., 2013). Rumen VFAs are the basis of de novo synthesis of fatty acids in the udder

(Palmquist, 2006), and changes in the milk fatty acids profile due to alterations in rumen biohydrogenation of polyunsaturated fatty acids, which occurs in the presence of low ruminal pH and may depress milk fat synthesis (Kleen and Cannizzo, 2012), could also affect milk coagulation properties. The nutrient content and technological properties of milk are critical issues for the dairy chain in areas where the majority of milk is processed into cheese, such as Europe. However, to our knowledge the effects of rumen acidity level on the technological cheese-making properties of milk have not yet been explored.

Starting from the hypothesis that a variation in rumen pH and the correlated rumen fluid composition are associated with RT and the technological properties of milk, this study aimed to explore whether dairy cows with different single point ruminal acidity status would differ in the pattern of rumination, and in the production, composition, coagulation properties and cheese yield (**CY**) of milk.

MATERIALS AND METHODS

Farms and Animals

The protocol of this study was compliant with the Italian legislation on animal care (DL n. 26, 4 March 2014). Collection of biological samples was performed by a skilled veterinarian under a sanitary inspection protocol of dairy herds aimed at monitoring rumen acidosis status on early lactating cows. In Italy, sanitary routine inspection, including collection of biological samples, does not require authorization, or an ID, or a protocol number. The study was conducted on 100 early-lactating Holstein cows (40 primiparous and 60 multiparous) kept in two commercial dairy herds in northern Italy. The farms, representative of the prevalent dairy system in the plains of the Veneto region, had an average herd size close to 150 lactating cows and an average milk yield close to 10,000 kg/cow/lactation. In both farms, cows were fed total mixed rations (TMRs), loosely housed in cubicle stalls, and milked twice a day, and their milk was destined for the production of typical hard cheeses. The composition of the TMRs and their nutritional contents, computed according to NRC (2001), are given in Table 1. The cows were fed ad libitum through a mixer wagon in a single distribution at around 07:00 h.

Table 1. Characteristics of the rations used in the two farms

Items[1]	Farm A	Farm B
Feed ingredients, kg dry matter (DM)/d:		
Corn silage	5.95	4.76
Ear corn silage	3.90	4.23
Barley silage	-	1.65
Grass silage	1.81	1.18
Meadow hay	2.30	0.88
Alfalfa hay	-	0.57
Wheat straw		0.44
Corn meal	0.87	-
Soybean meal (Solv. Extr.)	2.46	2.20
Sunflower meal (Solv. Extr.)	1.35	0.90
Commercial feed mixture	0.87	2.22
Propylene glycole	0.40	-
Linseed seed	0.35	-
Flaked soybean seeds	0.35	-
NaCl	0.05	0.05
$NaHCO_3$	0.05	0.05
Hydrogenated soybean oil	0.05	-
Total dry matter intake	20.76	19.18
Chemical constituents, g/kg DM:		
Crude protein	169	168
NDF	372	394
ADF	218	234
Starch	230	236
Ether extract	37	28
Ash	34	39
Net energy for milk, MJ/kg DM	6.9	6.3

[1]DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre.

Samples were collected once from 10 different cows in each of the 10 different recording sessions (6 in one herd and 4 in the other), so 100 cows were sampled during the study. At the beginning of each sampling session, the health status of the cows in the first 80 days in milk (DIM) was assessed on the basis of rectal temperature, heart rate, respiratory profile, appetite and faecal consistency. From these, 10 cows, primiparous and multiparous, with no obvious signs of clinical disease, were randomly selected for sampling.

Experimental Procedures

In each recording session, the cows were monitored for RT through external sensors over 5 consecutive days. Rumen fluid was collected once from each cow through rumenocentesis on the 3rd day of the recording session. On the same day as the rumenocentesis, the cows' milk yield was recorded, a milk sample was collected and a body condition score (**BCS**), evaluated according to Edmonson et al. (1989) on a scale ranging from 1, very thin, to 5, very fat, in 0.25 increments, was assigned by a skilled university technician having vast experience on scoring dairy cows for experimental purposes (Gallo et al., 2017).

Rumination Time

The RT was recorded with RuminAct™ (Milkline®, Podenzano, Italy), a microphone-based rumination monitoring system able to record the sounds of regurgitation and rumination. The system was used in previous research to measure the time spent ruminating during the day by dairy cows (Schiavon et al., 2015). The RuminAct™ microphone was fitted to the left side of the neck of each cow by a collar, and counted the minutes spent ruminating in 2-h intervals (min/120 min) from 00:00 to 24:00 h. Individual data were used to compute the circadian pattern of RT, expressed as the RT for each 2-h interval from 00:00 to 24:00 h (min/120 min, 60 records/cow) (Schiavon et al., 2015).

Rumen Fluid Sampling and Analysis

Rumen fluid (20 mL) was collected using a 50 mL syringe and a 13G 105-mm needle (Intralune PP, Vygon, France) inserted into the ventral sac of the rumen (Morgante et al., 2007). Rumen sampling procedures started around 5 hours after feed distribution, the recommended sampling time-point for ruminal fluid collection (Humer et al., 2018). The pH of the rumen fluid was determined immediately after sampling, using a portable digital pH meter (Zetalab PC70; XS instruments, Padova, Italy). An aliquot of 8 mL of rumen fluid was immediately acidified with 2 mL of hydrogen chloride (HCl 0.6 M) and refrigerated at 4 $^{\circ}$C until the samples arrived at the laboratory, where they were stored at -80^{o}C until analysis. The VFA and lactic acid contents were measured on the supernatant of the rumen fluid samples obtained by centrifugation (1300× g for 15 min) using an HPLC Perkin Elmer Series 10, mobile phase H2SO4 0.0025 N, flux 0.6 mL/min, detector Waters 410, column Gecko 2000 at a working temperature of 60^{o}C (Morgante et al., 2007).

Milk Sampling and Analysis

Milk samples (100 mL) were taken from each cow during the morning milking and stored in a refrigerator at -20^{o}C until analysis. Due to problems that occurred during the refrigeration of the samples, the composition and technological properties of the milk of 76 cows were determined. Milk composition (fat, protein, casein, lactose and total solids, respectively) was measured with a Milkoscan FT2 infrared analyser (Foss Electric A/S, Hillerød, Denmark). Milk pH was measured using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Somatic cell count (SCC) was obtained with a Fossomatic Minor FC counter (Foss Electric A/S). Milk coagulation properties (MCPs) and CY were assessed in duplicate for each cow according to the 9-MilCA method (Cipolat-Gotet et al., 2016) using 2 computerized lactodynamographs (Formagraph; Foss Electric A/S). The milk samples (9 mL) were placed in a Formagraph rack (8 samples per rack), heated to 35^{o}C for 15 min and mixed with 0.2 mL of rennet solution (Hansen Standard 215, 215 IMCU/ mL with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted in distilled water (1.2 % wt/vol). The rack was moved to the lactodynamograph and observed for 30 min (120 curd firmness measures taken from each milk sample, 1 every 15 s) to measure traditional MCP traits (RCT, k_{20} and a_{30}) and to obtain curd firming equation parameters (RCT_{eq}, k_{CF} and CF_P) according to Bittante (2011). The gelated milk samples were double-cut and heated for 30 min to 55^{o}C. The whey was drained from the curd, and analyzed for fat, protein, lactose and total solids content using FT2 (Foss Electric A/S, Hillerød, Denmark). The energy content of the milk and the whey was calculated as proposed by the NRC (2001). Three CY traits (CY_{CURD}, CY_{SOLIDS} and CY_{WATER}) and 4 milk nutrient recovery traits in the curd (REC) traits ($REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS} and REC_{ENERGY}) were determined from the weight and composition of the milk and the whey.

Editing Procedures and Statistical Analysis

A multivariate factor analysis was conducted to analyze the rumen fluid traits, including as variables the molar concentrations of acetate, iso-butyrate, normal-butyrate, propionate, isovalerate, normal-valerate, lactate, and the pH. The original variance of each trait was decomposed into its common and unique components, named as communality (BB') and uniqueness (Ψ), using the SAS FACTOR procedure (SAS Institute, Inc., Cary, NC, USA), as detailed by Mele et al. (2016). The number of latent explanatory factors to be extracted was based on the Eigen values (> 1), reliability in terms of relationships with the original variables, and the amount of explained variance. Factor reliability was improved through VARIMAX rotation. A variable was considered associated with a specific factor if the absolute value of its loading was ≥ 0.60. Two latent factors were extracted (Table 2): factor 1 was positively associated with acetate (load = 0.85, where load expresses the correlation between the latent factor and the measured trait), propionate (load = 0.75), iso-butyrate (load = 0.75), and lactate (load = 0.60), and negatively with pH (load = −0.78); factor 2 was positively related to normal-butyrate, iso-valerate and normal valerate. Factor 1 was associated with rumen acidity and used to compute a rumen acidity score for each cow, according to the following formula (Mele et al., 2016):

$$X' = y' \times (BB' + \Psi)^{-1} \times B$$

where x' is the row vector of the factor 1 scores, y' is the row vector of standardized traits [(value - mean)/standard deviation)], and B represents the corresponding loading elements of the BB' matrix of the theoretical factor variance model.

Table 2. Rotated factor pattern and proposed factor names

	Factor 1 "Rumen acidity"	Factor 2 "Protein degradation"	Communality	Eigen value
Acetate	0.85	0.15	0.75	1.19
Propionate	0.75	0.25	0.62	0.62
Iso-butyrate	0.73	0.32	0.63	0.85
Lactate	0.60	0.01	0.36	0.22
Normal-butyrate	0.49	0.69	0.71	0.49
Iso-valerate	0.36	0.71	0.63	0.29
Normal-valerate	-0.04	0.93	0.86	0.25
pH	-0.78	-0.33	0.72	4.10
Cumulative variance	0.60	0.40		

On the basis of the quartile of the rumen acidity score (QRA), the cows were classified in four groups having the same number of observations and ranging from the greatest to the lowest rumen acidity level moving from the 1st to the 4th QRA. The records were also classified for herd-test date (**HTD**, 10 classes, 10 cows/class), parity (**PAR**, primiparous and multiparous, 40 and 60 cows, respectively), and DIM (2 classes, DIM ≤ 35, 46 cows, and DIM > 35, 54 cows). Records relating to the technological properties of the milk were also classified for the position of the vat in the formagraph racks (8 vats per rack, a total of 16 classes). After editing for missing values, the final data set for RT included 3096 records and 52 cows. Records of RT (min/120 min) were classified according to the day of observation (**DAY**, 5 classes from the day 1 to day 5) and for the 2-h daily time interval of collection within each DAY (**HOURS**, 12 intervals from 00.00 to 24.00 h).

Data were analyzed using a mixed model procedure (SAS 9.4), which included different effects according to the trait category being analyzed, namely:

- for rumen fluid data (lactic acid and VFA content and proportion): the random effect of HTD and the fixed effects of PAR, DIM and QRA;

- for BCS, milk yield and milk composition data: the random effect of HTD and the fixed effects of PAR, DIM, and QRA;

- for the technological properties of milk (MCP and CY, for which 2 replicates per cow were available): the random effects of HTD and cow, and the fixed effects of PAR, DIM, vat, and QRA.

Polynomial contrasts ($P < 0.05$) were estimated between the least squares means of QRA in order to examine the response curve of the data with changing rumen acidity; the first-order comparisons measured linear relationships, whereas the second- and third-order comparisons measured quadratic and cubic relationships, respectively.

The circadian evolution of RT (min/120 min) was analyzed using the SAS MIXED procedure and a repeated measures model to allow for heterogeneous variances and correlations among different days. The model included the random effects of HTD and cow and the fixed effects of PAR, DIM, QRA, HOURS, DAY, and the QRA × HOURS and HOURS × DAY interactions. The analysis was carried out using compound symmetry as covariance structures, as it provided the least Akaike's Information Criteria (Littell et al., 1998). As the QRA × HOURS interaction was significant ($P = 0.04$), polynomial contrasts were estimated between the least squares means of the interaction in order to examine the response curve of RT within HOUR with changing rumen acidity.

RESULTS

Rumen Fluid Characteristics

As expected, rumen fluid pH was related to QRA and linearly decreased moving from the 4th to the 1st quartile (Table 3), characterized by a mean rumen fluid pH of 6.42 and 5.61, respectively. Nearly 32% and 14% of cows had a rumen fluid pH < 5.8 and < 5.6, respectively (data not in table). The total VFA content in the rumen fluid linearly increased moving from the 4th to the 1st quartile ($P < 0.001$), and was 40% greater in cows in the 1st than in those in the 4th QRA. In addition, the lactate content was influenced by QRA ($P = 0.003$), and reached the highest average value in cows of the 1st QRA, characterized by the highest rumen acidity. Acetate and propionate concentrations in the rumen fluid, which averaged 58% and 24% of total VFA, respectively, were significantly affected by QRA. Namely, the proportion of acetic acid linearly decreased and that of propionic acid linearly increased moving from the 4th to the 1st quartile ($P < 0.001$). As a consequence, the C2 to C3 ratio also decreased linearly as QRA decreased ($P < 0.001$), with average values close to 3 in the 4th and close to 2.2 in the 1st quartile, respectively.

Table 3. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on pH, lactate and volatile fatty acids (VFA) content and proportion of rumen fluid of Holstein cows

Items	Quartile of rumen acidity				SEM	*QRA*, *P* value	Contrasts, *P* value		
	QRA1	QRA2	QRA3	QRA4			Linear	Quadratic	Cubic
pH	5.61	5.82	6.04	6.42	0.05	<0.0001	<0.0001	0.17	0.92
Lactate, mmol/l	0.974	0.201	0.178	0.003	0.221	0.01	0.003	0.17	0.35
VFA, total, mmol/l:	98.54	86.57	77.01	62.74	2.09	<0.0001	<0.0001	0.49	0.31
Acetic acid	54.48	48.28	44.90	39.06	0.85	<0.0001	<0.0001	0.82	0.13
Propionic acid	25.84	21.56	18.28	13.13	1.07	<0.0001	<0.0001	0.65	0.49
Iso-butyric acid	0.77	0.63	0.49	0.40	0.03	<0.0001	<0.0001	0.34	0.71
N-butyric acid	13.02	12.06	9.92	7.65	0.75	<0.0001	<0.0001	0.17	0.60
Iso-valeric acid	1.88	1.58	1.36	1.13	0.15	<0.0001	<0.0001	0.74	0.84
N-valeric acid	2.55	2.43	1.86	1.70	0.35	0.002	<0.0001	0.94	0.29
C2:C3 ratio	2.23	2.32	2.60	3.01	0.13	<0.0001	<0.0001	0.16	0.92
VFA, mmol/100 mol:									
Acetic acid	55.79	56.05	58.73	61.37	1.2	<0.0001	<0.0001	0.14	0.47
Propionic acid	26.00	24.95	23.55	20.94	0.86	0.0004	<0.0001	0.33	0.81
Iso-butyric acid	0.78	0.73	0.63	0.66	0.04	0.02	0.005	0.28	0.28
N-butyric acid	13.05	13.87	13.01	12.04	0.72	0.12	0.51	0.66	0.12
Iso-valeric acid	1.88	1.81	1.79	1.79	0.17	0.95	0.63	0.78	0.99
N-valeric acid	2.56	2.74	2.43	2.83	0.36	0.27	0.52	0.49	0.08

__Rumination Time__

Rumination time exhibited a clear circadian variation (Figure 1), with two peaks: one at 10.00 to 12.00 h and one at 18.00 to 20.00 h. On average, cows spent nearly 375 min/day ruminating. QRA did not affect the average RT during the day (P > 0.05), even though it nominally increased as QRA decreased, and was nearly 8% greater in cows in QRA1 compared to those in QRA4 (data not shown in table). Conversely, we found a significant QRA–HOURS interaction (P = 0.04), that suggests an influence of rumen acidity on the circadian rumination pattern. Namely (Figure 1), RT linearly increased moving from the 4th to the 1st quartile during the daily time interval 08:00 to 12:00 (P < 0.05), but was similar across different QRA during the rest of the day.

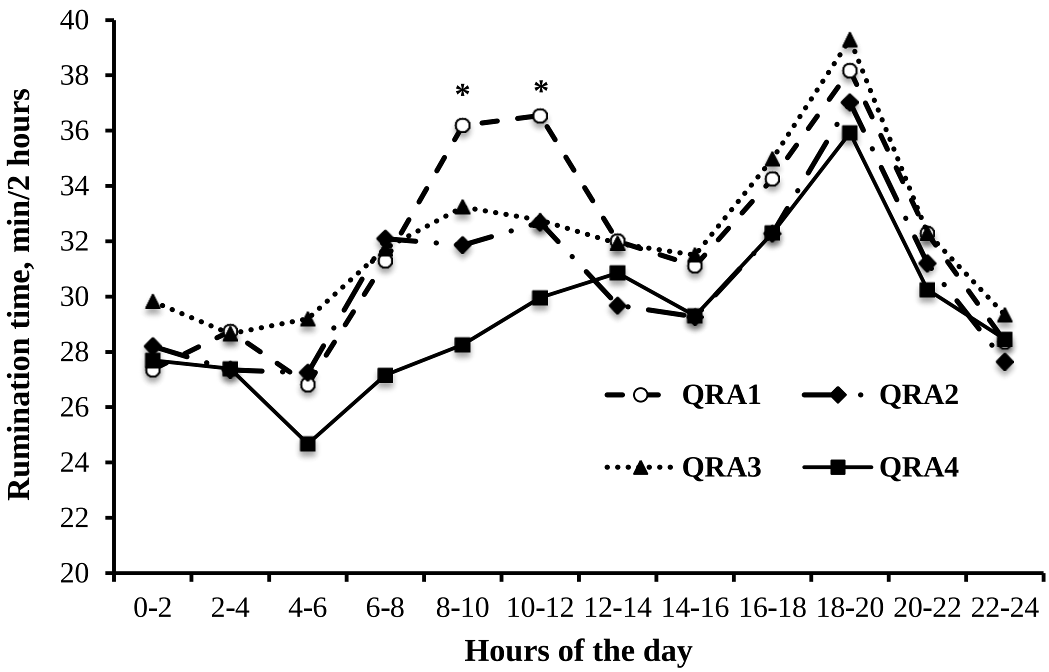

Figure 1. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on the time spent in rumination (min/2 h) during the day by lactating Holstein cows (P value of contrast relative to linear effect of QRA: * < 0.05; SEM ranged from 1.81 to 1.85 min/2 h).

Body Condition Score, Milk Yield and Milk Composition

Milk yield averaged 35.8 kg/d and linearly decreased as rumen acidity increased ($P <$ 0.03, Figure 2), so cows in the 1st quartile produced nearly 4.5 kg/d less milk than those in the 4th quartile. Conversely, QRA did not affect the cows' BCS, which was on average close to 3 regardless of the level of rumen acidity (Table 4). The fat and protein content of milk approached 3.20 and 3.05%, respectively, and were also not influenced by QRA. Additionally, the somatic cell score appeared unaffected by QRA.

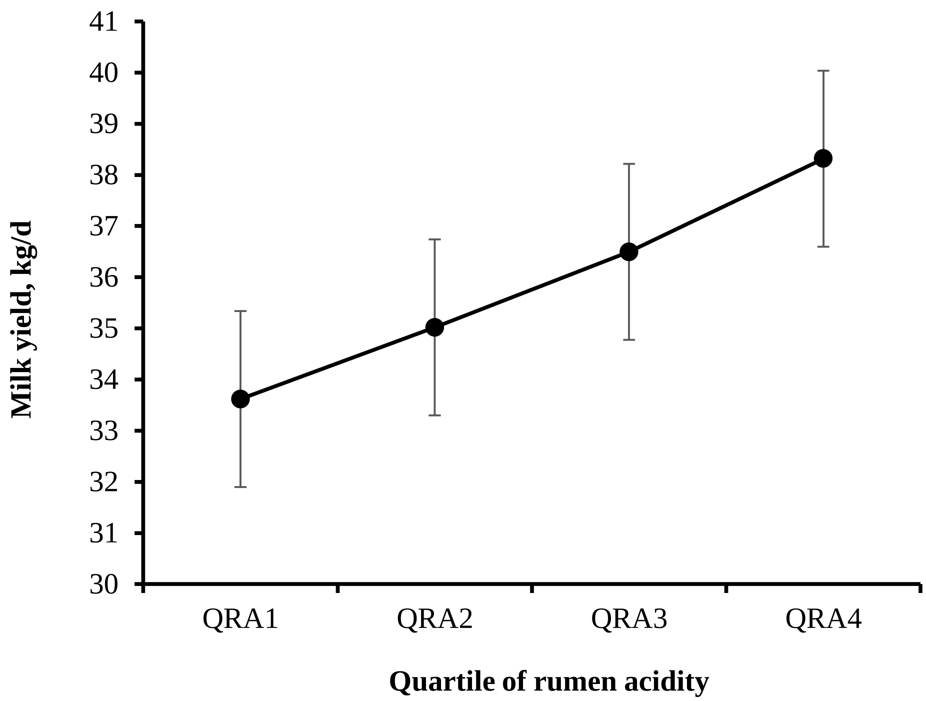

Figure 2. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on milk yield (*P* of the linear component: < 0.03).

Table 4. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on body condition score (BCS) and milk composition of Holstein cows

Items	Quartile of rumen acidity				SEM	*QRA*,
	QRA1	QRA2	QRA3	QRA4		*P* value
BCS	2.92	2.97	2.97	2.93	0.07	0.91
Milk pH	6.54	6.55	6.49	6.53	0.02	0.18
SCS[1]	3.83	4.21	3.18	3.48	0.59	0.41
Fat, %	3.26	3.05	3.10	3.31	0.16	0.62
Protein, %	3.05	3.01	3.17	2.96	0.07	0.14
Lactose, %	5.03	5.03	5.08	5.05	0.05	0.75
Total solids, %	11.96	11.74	12.01	11.95	0.16	0.66
Fat : Protein ratio	1.08	1.01	0.99	1.13	0.06	0.25

[1]SCS = $\log_2$ (SCC/100,000) + 3.

Milk Coagulation Properties and Cheese Yield Traits

On average, the rennet coagulation time (RCT) was close to 20.1 min. A curd firmness of 20 mm (k_{20}) was attained after almost 6.0 min from gelation, and the curd firmness 30 min after rennet addition (a_{30}) was 21.6 mm (Table 5). The mean values of the curd firming parameters were 19.2 min for the coagulation time calculated on the basis of all data points available (RCT_{eq}); 40.1 mm for the asymptotic potential curd firmness theoretically achievable at infinite time in the absence of curd syneresis (CF_P); and 7.6 %/min for the instant rate constant of curd firming (k_{CF}). None of the traits relating to the coagulation properties of milk differed across different QRA.

Table 5. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on single point milk coagulation properties (MCP) and curd firming equation parameters of Holstein cows

Items	Quartile of rumen acidity				SEM	*QRA*, *P* value
	QRA1	QRA2	QRA3	QRA4		
Single point MCP[1]:						
RCT, min	19.12	20.39	18.39	21.10	1.31	0.39
k_{20}, min	6.33	5.53	4.76	5.41	0.80	0.50
a_{30}, mm	24.72	22.30	29.34	21.15	4.01	0.35
Curd firming parameters[2]:						
RCT_{eq}, min	18.60	20.54	18.58	21.25	1.14	0.25
CF_p, mm	38.85	35.56	38.26	42.52	4.48	0.68
k_{CF}, % / min	12.33	12.13	14.15	10.70	0.95	0.09

[1] Single point MCP: RCT = rennet coagulation time; k_{20} = time to a curd firmness of 20 mm; a_{30} = curd firmness after 30 min from rennet addition.

[2] Curd firming parameters: RCT_{eq} = rennet coagulation time estimated using the equation; CF_P = asymptotic potential curd firmness; k_{CF} = curd firming instant rate constant.

On average, milk samples yielded nearly 14.4% of fresh cheese, comprising 36% solids and 64% water (Table 6). Milk protein, fat, solids and energy recoveries in the fresh cheese averaged 78, 64, 43 and 54%, respectively. QRA did not affect cheese yield or recovery traits.

Table 6. Effect of the quartile of rumen acidity (QRA1, greatest rumen acidity, to QRA4, lowest rumen acidity) on cheese yield (CY) and milk nutrient and energy recovery in curd (REC)

Items	Quartile of rumen acidity				SEM	*QRA*, *P* value
	QRA1	QRA2	QRA3	QRA4		
CY[1], %:						
CY_{CURD}	14.70	13.98	14.59	14.27	0.43	0.56
CY_{SOLIDS}	5.09	5.07	5.16	4.78	0.18	0.44
CY_{WATER}	9.49	8.96	9.45	9.52	0.36	0.58
REC[2], %:						
$REC_{PROTEIN}$	78.79	78.14	77.83	78.59	0.55	0.99
REC_{FAT}	66.68	63.86	61.68	60.17	4.08	0.47
REC_{SOLIDS}	43.46	42.92	41.60	40.83	1.02	0.68
REC_{ENERGY}	54.57	53.84	54.22	52.48	1.61	0.80

[1] CY_{CURD} = fresh cheese yield; CY_{SOLIDS} = total solids cheese yield; CY_{WATER} = water retained in the curd.

[2] $REC_{PROTEIN}$ = milk protein retained in the curd; REC_{FAT} = milk fat retained in the curd; REC_{SOLIDS} = total milk solids retained in the curd; REC_{ENERGY} = milk energy retained in the curd.

DISCUSSION

Rumen Parameters and Rumination Time

Ruminal acidosis is a nutritional disorder of high-producing dairy cows, particularly in early lactation, when the energy density of diets is increased to meet their high nutritional requirements, and feeding excessively fermentable diets increases the risk of depressed rumen pH. The diagnosis of ruminal acidosis is mainly dependent on the monitoring of rumen pH, so rumenocentesis is usually performed in field trials to collect rumen fluid samples (Enemark, 2009; Kleen and Cannizzo, 2012). However, diagnosis is difficult in farm conditions as ruminal pH is subject to daily fluctuation and a single time-point measurement is not accurate enough to assess long lasting pH depression (Danscher et al., 2015; Humer et al., 2018). Given these difficulties, rather than referring to the possible and questionable SARA condition, in this study we combined the single point measures of the rumen fluid pH and VFA composition in a latent variable representing rumen acidity and we used this variable to investigate the relationships between the rumen acidity level and RT, composition and technological properties of milk.

Nearly 32% of cows had a rumen pH lower than 5.8, and in nearly 14% it was lower than 5.6. These values are consistent with data from field surveys where cows were sampled once for ruminal fluid (Morgante et al., 2007; Plaizier et al., 2008; Kleen et al., 2013). The total VFA content increased linearly moving from the 4th to the 1st QRA, confirmation that the depression of rumen pH was mainly the consequence of an increased production of VFA in the rumen and reduced absorption of VFA (Plaizier et al., 2008). Moreover, the increase in rumen acidity was associated with a linear decrease in the concentration of acetate and with a linear increase in propionate in the rumen fluid, the consequence of a variation in the cellulolytic and the amylolytic bacterial activity due to change in the ruminal acidity condition (Lessire and Rollin, 2013). These variations may have a negative effect on the de novo synthesis of fatty acids (≤ 16 chain carbons) in the udder, because acetate is the primary precursor of fat synthetized by the mammary gland (Palmquist, 2006).

Plaizier et al. (2008) stated that ruminal pH affects the growth of microbial populations and the physiological functions of the rumen, and it is also possible that greater rumen acidity would be related to different RT. In fact, differences in RT can affect saliva secretion, which seems to be greater during eating than during resting (Maekawa et al., 2002) and helps to stabilize ruminal pH by buffering the organic acids produced during the fermentation of carbohydrates (Beauchemin, 2018). There is growing interest in using ruminating behavior as a tool for early identification of health problems in dairy cows (Schirmann et al., 2016), although little is known about the relationship between rumen acidity and RT. The average daily RT and the circadian RT pattern observed in this study are similar to the figures and patterns reported by Schiavon et al. (2015) for cows fed on similar rations and monitored using the same sensor. In the current research, we found that the average daily RT seems to be unaffected by differences in rumen acidity. However, the circadian pattern of RT was influenced by QRA, and the time spent ruminating increased linearly at increasing rumen fluid acidity after the morning feeding, when ruminal pH is expected to drop to its lowest value. DeVries et al. (2009) observed that cows spent less time ruminating on the first day following an acidosis challenge, but more time on the second day and returned to pre-acidosis challenge levels thereafter. In a subclinical acidosis challenge trial, Khiaosa-ard et al. (2018) observed that cows susceptible to SARA had longer ruminating and total chewing times compared with tolerant cows. Increased RT could, therefore, be interpreted as a sign of a cow's resilience and a way to counterbalance the decrease in rumen acidity with increased saliva production.

BCS, Milk Yield and Composition

Subclinical acidosis has been associated with a lower and erratic feed intake (Mulligan and Doherty, 2008), which may affect the cows' BCS, although we did not find any relationship between QRA and BCS. Our results are in agreement with the findings of O'Grady et al. (2008) and Bramley et al. (2013), who also scored the cows' condition at rumen fluid collection. It may be that a single time-point approach is not appropriate for traits that reflect long-term variation dynamic, such as BCS. Kleen et al. (2009) also failed to find any significant relationship between the cows' ruminal pH and their BCS measured once at rumenocentesis, but they observed that cows with SARA exhibited a greater fall in BCS over the calving period.

The association between depressed rumen pH and milk production traits is rather controversial in the literature. In the current study, we found a strong communality among pH and some VFAs, but it is worth noting that pH explained only part of the total variance of the rumen acidity latent factor. In the current study, greater rumen acidity was related to a significant reduction in milk yield, and the magnitude of the response was similar to that reported by Stone (1999), who found the milk yield of cows with SARA to be nearly 3 kg/d lower than that of normal cows. Conversely, in a field study carried out by O'Grady et al. (2008), milk yield was found to be unaffected by rumen pH. Similarly, Kleen et al. (2013) found no association between milk yield and the rumen pH in a survey of German herds, and Danscher et al. (2015) did not find cows with induced SARA to have a lower milk yield.

Milk nutrient content appeared to be independent of rumen acidity in our study, despite the fact that a reduction in the acetate to propionate ratio in the rumen could result in the depressed synthesis of fat in the mammary gland (Lessire and Rollin, 2013). Depressed ruminal pH has been frequently associated with a lower milk fat content (Enemark, 2009; Humer et al., 2018). Experimentally-induced subclinical acidosis reduced the milk fat percentage in some studies (Danscher et al., 2015), but not in others (Gao and Oba, 2014). Inconsistent results with respect to milk fat reduction after an acidosis challenge have been ascribed to the bouts of low rumen pH being too short to affect the milk fat content (Gao and Oba, 2014). However, field studies have also frequently shown inconsistent relationships between rumen pH and milk fat content (O'Grady et al., 2008; Kleen et al., 2013), and in a review of data from field studies, Kleen and Cannizzo (2012) reported that milk fat depression was not prevalent in herds or cows with SARA.

Milk Coagulation Properties and Cheese Yield

Milk coagulation properties are commonly assessed using a lactodynamography to record three single-point measures (RCT, $k_{20,}$ and a_{30}) useful to the dairy industry (Bittante, 2011). Nearly 10% of the samples in our study failed to coagulate within 30 min of rennet addition, a figure comparable to the proportion of non-coagulating samples reported by Cecchinato et al. (2011) in a large survey carried out on Holstein cows. The average RCT and k_{20} we observed were similar to those found by Malchiodi et al. (2014) in purebred Holstein cows' milk, whereas our a_{30} estimates were lower. Although widely used for assessing the technological traits of milk, traditional MCP parameters have certain limitations, such as the incidence of samples that do not coagulate within 30 min, precluding estimation of the RCT, k_{20} and a_{30}, and the incidence of late-coagulating samples, precluding the estimation of k_{20} (Bittante, 2011). These limitations are partly overcome by a new procedure proposed by Bittante (2011), in which the curd-firming process is modeled over time, so even samples with very late coagulation or very slow curd firming can be analysed.

Neither the traditional MCP traits nor the curd-firming parameters estimated using the modeling equation were affected by QRA in our study, even though milk technological properties are influenced by several factors that may be related to ruminal conditions. It is well known that MCPs depend on milk composition, and that fat content is one of the main factors influencing the technological ability of milk (Troch et al., 2017). Milk pH, too, may impact the technological properties of milk (Stocco et al., 2015). However, neither milk composition nor milk pH were affected by ruminal acidity in our study, and this can at least partly explain the lack of relationships found between MCPs and ruminal acidity. Moreover, Bittante et al. (2015) and Stocco et al. (2017) found some differences in the RCT and curd firming patterns between the milk produced by low-input herds and that produced by more intensive herds, which may also differ in the amounts of concentrates fed to the cows and consequently in rumen fluid conditions. The extent to which these findings may be related to rumen acidity is unknown. The relationships between rumen fluid acidity and the technological characteristics of milk have not been explored yet, and further research in this area is needed.

The efficiency of the cheese-making process relies heavily on CY as well as on the recovery of individual milk constituents in the curd and their loss in the whey. Cheese yield traits are influenced by several dairy cow-related factors, such as breed, milk composition and MCPs, and farming conditions (Cipolat-Gotet et al., 2013), but there has been little investigation so far of the effects of common health disorders on CY, particularly at individual cow level. This is relevant for conditions that seem to be widespread in commercial herds, such as those related to an increase in rumen fluid acidity. The average fresh CY (14.3%) we found is comparable to the 14.1% CY predicted by Cecchinato et al. (2015) for Holstein Friesian cows, and both studies had similar recoveries of protein in the curd, although we found a greater proportion of water to solids in the curd, resulting in lower recoveries of fat and solids. Neither cheese yield nor nutrient and energy recoveries were influenced by rumen fluid acidity, even though the cows in the bottom QRA, characterized by an average rumen fluid pH close to 5.6, produced milk with a nominally greater CY and nutrient recovery in cheese than cows in the other QRA.

CONCLUSIONS

The results of this study confirm that the level of rumen acidity in early-lactating Holstein cows with no obvious signs of clinical disease may be quite variable, at least in intensively-managed commercial dairy herds. The decrease in rumen pH has been associated with a linear increase in the content of all VFAs in the rumen fluid and with changes in the relative proportions of acetate and propionate, so a latent variable was extracted to represent the overall rumen acidity condition. Cows with different ruminal acidity spent a similar amount of time ruminating during the day, but their RT differed after the morning feeding. The time spent ruminating increased during the 08:00 to 12:00 h interval as rumen acidity increased. However, the usefulness of monitoring RT as a tool for identifying cows with acid rumen conditions needs to be revaluated with a larger number of cows. Even when there were differences in rumen acidity, the composition, coagulation, curd firming patterns and cheese yield of milk were unaffected, excluding a possible detrimental relationship between acid rumen fluid conditions and the properties of milk destined for cheese production. As the current study was carried out in field conditions, the single time-point rumenocentesis used to collect rumen fluid samples was unfit to measure the amount of time spent daily under the critical pH value, thus preventing the possibility of the reliable diagnosing of an eventual state of subacute ruminal acidosis. Therefore, further studies are needed to investigate more thoroughly the relationships between rumen pH depression and the technological properties of milk.

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CHAPTER 2

Effects of summer transhumance of dairy cows to alpine pastures on body condition, milk yield and composition, and cheese making efficiency

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ABSTRACT

Summer transhumance to alpine pastures (**ALP**) is widespread in dairy systems of alpine regions. This study aimed to investigate the effects of transhumance of Brown Swiss cows to ALP on the yield, composition and coagulation properties of milk (**MCP**), and on cheese yield (**CY**). The study involved 12 multiparous cows kept on a mountain lowland permanent farm (**PF**), which were divided into two equal groups: one remained on the PF, the other was moved to the ALP (1860 m asl) from July to September. Every month (June to October) daily milk yield (**MY**) and body condition score (**BCS**) were recorded, and individual milk samples (n = 60, 2000 mL each) were collected to assess milk composition, MCP and CY. Compared with PF, ALP cows had a reduced MY and BCS, which was maintained on return to the PF, greater fat and lower protein contents of milk. Neither MCP nor CY were affected by summer transhumance. In conclusion, summer transhumance did not affect the cheese making efficiency of milk, but depressed MY and consequently daily cheese yield, which was nearly 2 kg/d lower for the ALP than the PF cows, and was only partially recovered after returning to the PF in autumn.

Keywords: dairy cow, summer transhumance, alpine pasture, milk yield, milk coagulation properties, cheese yield

INTRODUCTION

The transhumance of dairy cattle to temporary farms on mountain pastures during the summer months is an important, long-standing, traditional practice in the alpine regions of several European countries (Mack et al., 2013; Sturaro et al., 2013; Fuerst-Waltl et al., 2019). The practice has economic advantages for the alpine dairy sector, as it provides additional forage supply and may increase the added value of the milk obtained, destined mostly for the production of high-value local cheeses (Bovolenta et al., 2009; Bergamaschi et al., 2016). Moreover, transhumance and the management of summer pasture farms provide society and citizens with several positive services (Leroy et al., 2018), by helping to preserve biodiversity and natural habitats (Marini et al., 2011; Battaglini et al., 2014), by contributing to tourism and recreational activities that help to preserve the traditional alpine landscape, cultural heritage, and established customs (Gandini and Villa, 2003; Kianicka et al., 2010; Daugstad and Kirchengast, 2013), and by helping to protect natural resources and the environment from natural hazards (Fuerst-Waltl et al., 2019). Lastly, dairy products from alpine pastures are presumed to be healthier, due to their favorable fatty acid profile (Coppa et al., 2011; Bergamaschi and Bittante, 2017), and are positively perceived by consumers for attributes related to taste, health, wholesomeness, and animal welfare (Gandini and Villa, 2003; Bergamaschi and Bittante, 2018).

When dairy cows are transhumed to summer alpine pastures, they undergo a number of changes compared with those that remain on permanent lowland farms, including different quality and availability of feedstuffs, regrouping of animals, adaptation to pastures, altitude-related hypoxia, and harsh climatic conditions (Leiber et al., 2006; Zendri et al., 2017). Changes intrinsic to the alpine conditions may affect the cows' nutritional status and metabolism, which in turn may influence milk yield, composition, and processing qualities. Several studies have evaluated the performance of cows and the characteristics of milk produced during summer transhumance, and a few of these have evaluated the animals and their milk before, during, and after summer transhumance (Zendri et al., 2017). However, studies comparing the cows kept on the lowland farms with those temporarily moved to highland pasture during the summer are scarce.

Based on these premises, this study aimed to investigate the response of Brown Swiss dairy cows to summer transhumance to alpine pastures in terms of the effects on body reserves, milk yield, composition, and coagulation properties, and individual cheese yield before, during and after transhumance compared with a lowland control group over the same period.

MATERIALS AND METHODS

Farms, Animals, and Sample Collection

The trial was carried out from June to October with a dairy herd kept on a lowland permanent farm (PF; Malè, Italy, 737 m above sea level), and cows transhumed to a temporary summer highland farm (ALP; Malga Juribello, Italy, 1860 m above sea level), both located in the Trento province in the Northeastern Alps.

The PF herd comprised 92 lactating Brown Swiss that were loose-housed, milked in a milking parlor, and fed meadow and alfalfa hay and compound feeds. Every year around the end of June, part of the herd is moved from the PF to the ALP, where the cows are free to graze day and night on a typical *Nardetum alpigenum* association pasture, which has replaced the native woodland and shrub land (Orlandi et al., 2000). The cows are moved around different areas of the pasture according to grass availability without a rigid rotation plan, and each is also given a compound feed supplement in the milking parlor.

According to these practices, in the current experiment, 12 mid-lactation multiparous cows were selected at the beginning of the trial (June) and allotted on the basis of their parity number and days in milk (**DIM**) to two treatments, one of 6 control cows, which remained on the PF throughout the trial, the other of 6 cows, which were transhumed to the ALP at the beginning of July, and back to the PF at the beginning of October. The cows in both treatments had similar

(*P* > 0.05, based on t-test) parity numbers (2.5 and 2.8, respectively) and days in milk (DIM, 143 and 120, respectively).

The cows kept on PF were fed meadow and alfalfa hay (8 to 10 and 2 to 3 kg/d per head, respectively) and a commercial mixture of cereals, soybean meal, linseed, and maize germ meal (8.0 ± 2.0 kg/d, according to milk yield). Net energy and crude protein contents of the commercial mixture fed in the PF were 7.2 MJ and 150 g per kg as fed, respectively. In the ALP, the cows were kept at pasture, and received a commercial mixture of corn, wheat barn, soybean meal, sugarcane molasses, minerals, and vitamins as supplement (5.0 ± 1.5 kg/d, according to milk yield) during the milking. Net energy and crude protein contents of the commercial mixture fed in the ALP were 7.1 MJ and 140 g per kg as fed, respectively.

The following data were recorded and samples collected from all cows each month from June to October, during which period cows of one treatment remained continuously on the PF, the others on the PF in June and October and on the ALP from July to September:

- individual daily milk yield (MY, kg/d);
- individual milk samples (2000 ml per cow) collected during the evening milking and immediately refrigerated at 4°C without preservative;
- BCS, assessed by a skilled operator on the same day as milk sample collection using the technique developed by Edmonson et al. (1989), and expressed on a scale from 1 (thin) to 5 (fat) in increments of 0.25.

When cows were alternatively on the PF or on the ALP (from July to September), the monthly collection of milk samples was carried out in two subsequent days. All milk samples were transferred to the milk laboratory of the Department of Agronomy, Food, Natural

Resources, Animals and Environment (DAFNAE) of the University of Padova and analyzed and processed the following morning within 20 h of collection.

Milk Quality Traits

Individual milk samples were analyzed for fat, protein, and lactose content with a Milkoscan FT2 infrared analyzer (Foss Electric A/S, Hillerød, Denmark) calibrated according to reference methods: ISO 8968-2/IDF 20-2 for protein, ISO 1211/IDF for fat, and ISO 26462/IDF 214 for lactose. Somatic cell count (SCC) was obtained with a Fossomatic Minor FC counter (Foss Electric A/S) and log-transformed to somatic cell score (SCS) as proposed by Ali and Shook (1980).

Milk Coagulation and Curd Firmness Properties

Curd firmness was measured on 120 milk samples (12 cows × 5 monthly samples × 2 replicates) every 15 seconds for 45 min (180 measures from each milk sample, in duplicate) using a lactodynamograph (Formagraph; Foss Electric A/S, Hillerød, Denmark) and according to the procedure described in detail by Stocco et al. (2017). In brief, a pendulum calibration was carried out before each session of the trial; 10 mL of milk was heated to 35°C, then mixed with 200 μL of rennet solution (Hansen Standard 215 with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) freshly diluted to 1.2% (wt/vol) in distilled water.

We considered the traditional single point parameters of milk coagulation properties (MCP) reported by McMahon and Brown (1982), namely, rennet coagulation time (RCT, min), defined as the time from rennet addition to milk gelation; curd-firming time (k_{20}, min), defined as the time from gelation to a firmness of 20 mm within 45 min of enzyme addition; and curd firmness 30 and 45 min after rennet addition (a_{30} and a_{45}, respectively, mm). In addition, we modeled all the curd firmness observations from each milk sample and estimated the individual curd-firming and syneresis equation parameters (Bittante et al., 2013), namely, RCT_{eq} estimated from the individual curd-firming equations, the curd-firming instant rate constant (k_{CF}), the curd syneresis instant rate constant (k_{SR}), maximum curd firmness value (CF_{max}), and time at CF_{max} (t_{max}).

Cheese-Making Procedure and Traits

To assess cheese-making properties, 60 individual 1500 mL samples (12 cows × 5 monthly samples) were processed according to the model cheese-making procedure described in detail by Stocco et al. (2018). Briefly, each milk sample was poured into a stainless steel laboratory-vat, placed in a water bath (5 vats in each of 3 water-baths), heated to 35°C (30 min), after which 8 mL of the same type of rennet used for the MCP analysis diluted to 4.29% in distilled water was added. After coagulation, the curd was cut, then drained for 30 min, and the resulting whey was measured for chemical composition using a Milkoscan FT2 infrared analyzer (Foss Electric A/S, Hillerød, Denmark). The curd was pressed for 30 min at 250 kPa by a cheese-pressing machine, turning every 10 min, then soaked in a brine solution (20% NaCl) for 30 min. After brining, the cheese wheels were weighed and pH measured with a Crison Basic 20 electrode (Crison Instruments SA, Barcelona, Spain). The following traits were computed from the weight and composition of the milk, whey, and curd: Three percentage cheese yield traits ($\%CY_{CURD}$, fresh cheese yield; $\%CY_{SOLIDS}$, total cheese solids yield; and $\%CY_{WATER}$, water retained in the curd); three percentage milk nutrient recoveries in the curd ($REC_{PROTEIN}$, milk protein retained in the curd; REC_{FAT}, milk fat retained in the curd; and REC_{SOLIDS}, total solids retained in the curd); and daily cheese yield (dCY, kg/d), computed by multiplying MY by $\%CY_{CURD}$ and expressed as a daily measure.

Statistical Analysis

All the data were analyzed according to a linear mixed model (SAS 9.4, SAS Institute, Cary, NC, USA) that included the fixed effects of the Month × Treatment combination (8 levels: 4 months, July to October, and 2 treatments, PF and ALP), and the random effect of cow within Month × Treatment. The value measured in June was included as a linear covariate in the model for each trait to correct for possible initial differences among cows. Polynomial contrasts were estimated between the 5 least square means of month within PF to examine the response curve of each trait (linear, quadratic, and cubic components) during the 5 months in the cows kept on PF as a measure of the effect of season and lactation advancement. Contrasts between PF and ALP treatments were estimated separately within each month to test for the effect of transhumance to highland pasture during the summer months (July, August, and September) and the residual effect after returning to the PF (October).

RESULTS

Table 1 shows the descriptive statistics and results of the mixed model for BCS, MY, composition, and MCP, and CY of cows kept on the PF or moved to the ALP. The average MY was 25.7 kg/d, and fat and protein contents averaged 3.89 and 3.78%, respectively. The samples coagulated 22 min after rennet addition (RCT), and a curd firmness of 20 mm (k_{20}) was obtained after 4.5 min. Average curd firmness at 30 min was 30 mm and increased to 42 mm at 45 min from rennet addition. On average, one kg milk yielded 160 g curd (66 g of solids, 94 g of retained water), producing 4.2 kg/d of curd. There were similar variations in MY and RCT, with coefficient of variation (CV) equal to 31 and 26%, respectively, whereas $\%CY_{CURD}$ had a much lower CV equal to 11%.

The combined Month × Treatment effect significantly affected most traits examined, as it combines the effects of advancing lactation within cow and changes in environmental conditions due to the advancement of the season with effects related to the different farming conditions. The covariate for the initial value (June) also reached statistical significance for several traits, allowing us to adjust the least squares means for possible differences among the cows at the beginning of the trial. Polynomial contrasts estimated between the least squares means of month for PF cows showed there were linear relationships between most traits and quadratic relationships between some traits with advancing season, but the cubic component never reached statistical significance.

The least squares means of the combined effects of treatment and month for all analyzed traits are plotted in Figure 1, Figure 2, Figure 3 and Figure 4. Each figure also includes the linear or quadratic trends of the traits observed in PF cows, where statistically significant, and asterisks indicate significant differences between the ALP and PF cows in a given month.

Table 1. Descriptive statistics (mean ± standard deviation) and results from the mixed model for body condition score (BCS), milk traits, milk coagulation properties (MCP), curd firming and syneresis modeling parameters (CFt), percentage cheese yield (%CY), milk nutrients recovery in curd (REC), and daily yield of curd (dCY_{CURD}) of cows kept on the permanent farm or moved to temporary summer pasture (Treatment) during the June to October interval (Month): F-values and significance (* = P < 0.05; ** = P < 0.01) for the combined Treatment × Month fixed effect, and for the covariate on the initial value of the trait (COV); percentage of variance explained by the random effect of cow on total variance (Cow); P-value of the linear (L), quadratic (Q) and cubic (C) trends of the trait from June to October within permanent farm (TREND)

Traits	Variables	Mean ± SD	Month × Treatment	COV	Cow	RMSE[1]	*P* value of TREND		
							L	Q	C
BCS and milk traits:	BCS	3.09 ± 0.21	5.83**	25.88**	2	0.14	0.39	0.70	0.39
	Milk yield, kg/d	25.71 ± 8.04	6.09**	4.51*	32	3.32	<.0001	0.07	0.37
	Fat, %	3.89 ± 0.72	4.70**	0.48	8	0.46	0.002	0.05	0.10
	Protein, %	3.78 ± 0.31	25.06**	5.63*	53	0.13	<.0001	0.06	0.81
	Lactose, %	5.01 ± 0.21	5.57**	6.53*	57	0.10	0.0005	0.38	0.50
	SCS	3.49 ± 1.62	2.72*	7.38*	36	1.03	0.002	0.32	0.95
Single-point MCP[2]:	RCT, min	21.99 ± 5.80	8.54**	13.95**	21	3.34	<.0001	0.002	0.64
	k_{20}, min	4.53 ± 1.57	5.65**	2.28	40	0.99	0.003	0.05	0.70
	a_{30}, mm	30.14 ± 16.44	8.11**	8.32**	26	9.43	0.002	0.01	0.57
	a_{45}, mm	42.43 ± 9.14	14.00**	0.19	40	5.12	0.002	0.01	0.79
CF_t parameters[3]:	RCT_{eq}, min	22.77 ± 5.70	9.47**	14.47**	20	3.21	<.0001	0.001	0.68
	k_{CF}, %/min	10.74 ± 3.14	3.12*	15.09**	25	2.19	0.004	0.72	0.20
	k_{SR}, %/min	0.96 ± 0.38	2.50*	10.35**	30	0.27	0.03	0.35	0.42
	CF_{max}, mm	46.29 ± 7.80	17.21**	1.29	46	4.02	0.001	0.001	0.76
	t_{max}, min	46.36 ± 8.94	3.90**	14.22**	23	6.00	0.001	0.12	0.19
%CY :	Curd	16.06 ± 1.82	2.37*	5.05*	17	1.46	0.03	0.72	0.18
	Solids in curd	6.62 ± 0.70	4.80**	0.54	24	0.44	0.001	0.06	0.11
	Water in curd	9.44 ± 1.42	2.40*	1.13	33	1.08	0.11	0.74	0.25
REC, %:	Fat	83.18 ± 4.35	0.69	1.43	19	0.64	0.23	0.82	0.84
	Protein	78.61 ± 1.67	2.98*	40.11**	54	0.41	0.37	0.19	0.08
	Solids	50.34 ± 3.39	6.88**	0.67	32	2.04	0.01	0.16	0.09
Daily yield of curd	dCY, kg/d	4.16 ± 1.30	3.81**	1.05	32	0.68	0.06	0.43	0.14

[1]RMSE= root mean squared error.

[2]Single-point MCP: RCT = rennet coagulation time; k_{20} = curd firming rate as min to a curd firmness of 20 mm; a_{30} (a_{45}) = curd firmness after 30 (45) min from rennet addition.

[3]Curd firming: RCT_{eq} = rennet coagulation time estimated from the equation; CF_P = asymptotic potential curd firmness; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness attained within 45 min; t_{max} = time to reach CF_{max}.

The least squares means of BCS are shown in Figure 1. At the beginning of the trial, in June, BCS averaged 3.11 and remained essentially unchanged over the months for cows kept on the PF, reaching an average of 3.26 in October. However, the cows moved to the ALP lost body reserves, so their BCS was lower ($P < 0.01$) in August and in October after returning to the PF than that of the cows that remained on the PF.

Over the period June to October, the milk yield and lactose content of PF cows (Figure 1) decreased linearly, whereas protein and fat content increased linearly and quadratically, respectively, as expected due to the advancing of the stage of lactation. Transhumance notably reduced the MY of the ALP cows, which was 30–35% lower ($P < 0.05$) during the three months on the alpine pasture than that of the PF cows. The milk of the ALP cows also had lower protein and lactose contents than the milk of the PF cows during this period, but a greater fat content in the first (+20%) and second (+35%) months on the alpine pasture. The differences in milk yield and composition between the cows of the two treatments were less evident in October after the return of the transhumant cows to the PF.

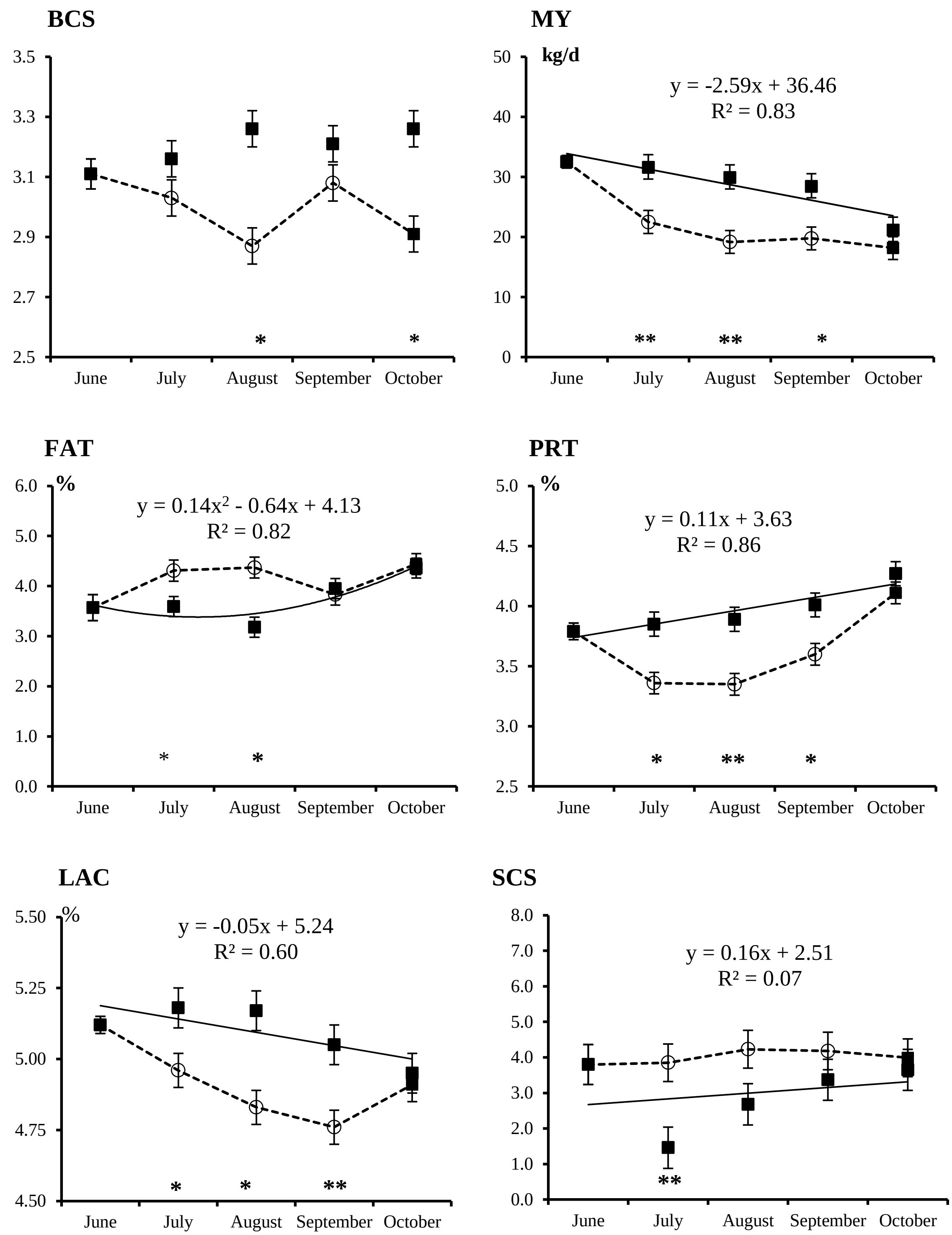

Figure 1. Least squares means and SE of the combined Treatment × Month effect for body condition score (BCS) and milk traits (milk yield – MY, content of milk fat – FAT, milk protein – PRT, and milk lactose – LAC, and somatic cell score – SCS) of Brown Swiss cows kept on the permanent farm (■ and continuous line) or moved to highland summer pasture from July to September (○ and dotted lines). Equations describe the trends of month within permanent farm, where statistically significant (R^2 is shown). Asterisks refer to significant differences between cows moved to highland summer pasture and those kept on the permanent farm in a given month (* $P < 0.05$ or ** $P < 0.01$).

The least square means of the combined Treatment × Month effect for single-point MCP are reported in Figure 2. In general, the MCP of milk from PF cows tended to improve in a quadratic pattern from June to October, with advancing lactation stage. The RCT and k_{20} decreased (improved), especially in the last two months of the trial, while curd firmness traits (a_{30} and a_{45}) increased in the same period. Transhumance did not affect single point MCP traits, which were nearly identical in the milk samples from both treatments for all months.

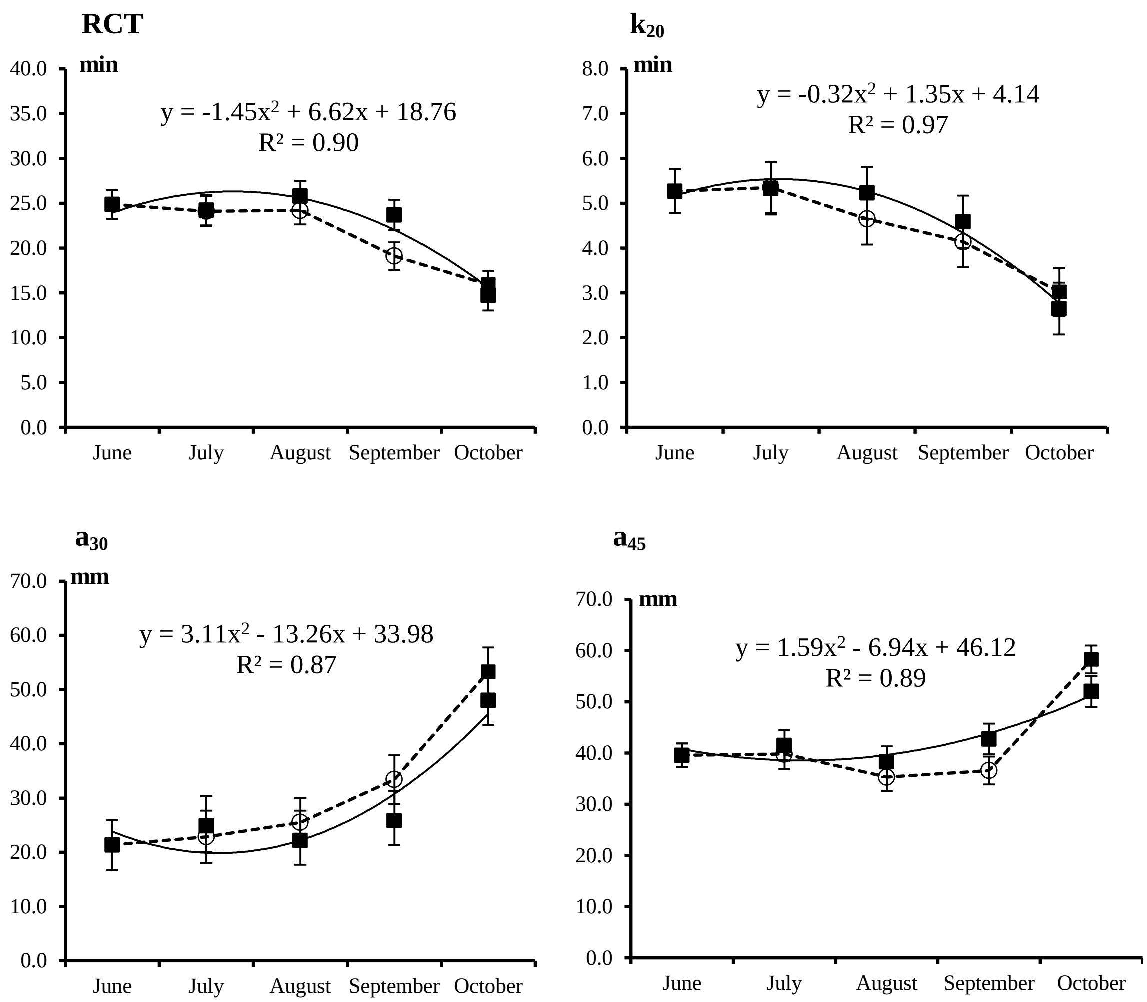

Figure 2. Least squares means and SE of the combined Treatment × Month effect for milk coagulation traits (rennet coagulation time – RCT, curd firming rate as time to a curd firmness of 20 mm - k_{20}, and curd firmness after 30 and 45 min from rennet addition - a_{30} and a_{45}, respectively) of Brown Swiss cows kept on the permanent farm (■ and continuous line) or moved to highland summer pasture from July to September (○ and dotted lines). Equations describe the trends of month within permanent farm (R^2 is shown). Differences between cows moved to highland summer pasture and those kept on the permanent farm in a given month were never significant ($P > 0.05$).

Least squares means of the combined Treatment × Month effect for the curd-firming model parameters are reported in Figure 3 and basically confirm the trends of the single-point MCP.

Namely, in PF cows, all CF_t parameters improved over the trial (and advancing stage of lactation), especially in September and October (quadratic trend) for RCT_{eq} and CF_{max}. Transhumance of cows to summer alpine pasture did not affect the curd-firming model parameters, which mostly matched those of the milk from PF cows, so that cows of the two treatments had a common pattern of milk coagulation, curd firming, and syneresis.

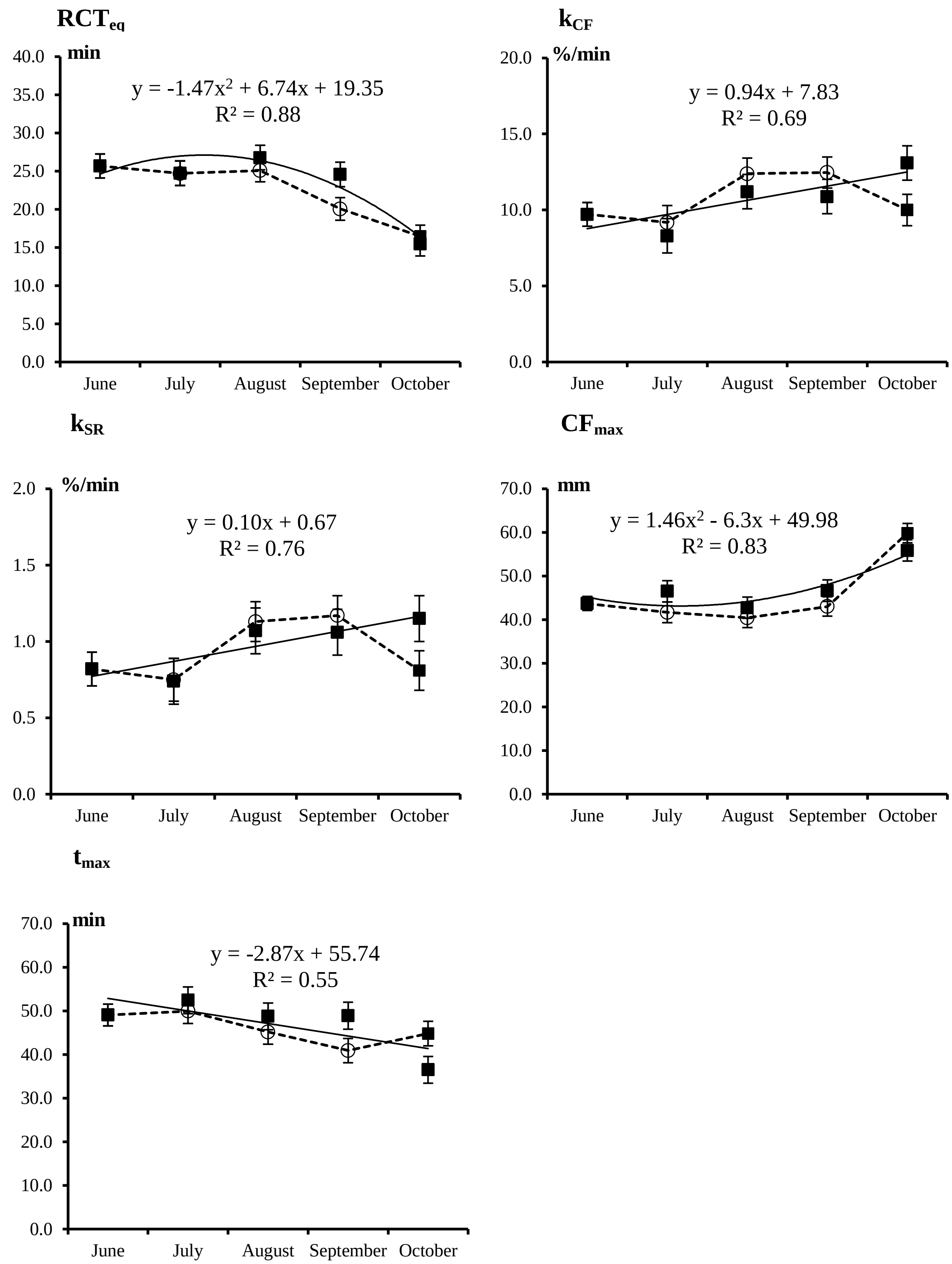

Figure 3. Least squares means and SE of the combined Treatment × Month effect for curd firmness model parameters (rennet coagulation time equation - RCT_{eq}, curd firming instant rate constant - k_{CF}, syneresis instant rate constant – ksr, maximum curd firmness - CF_{max}, time to reach CF_{max} - t_{max},) of Brown Swiss cows kept on permanent farm (■ and continuous line) or moved to highland summer pasture from July to September (○ and dotted lines). Equations describe the trends of month within permanent farm (R^2 is shown). Differences between cows moved to highland summer pasture and those kept on the permanent farm in a given month were never significant ($P > 0.05$).

Least squares means of the combined Treatment–Month effect for cheese yield and milk nutrient recovery in the curd are shown in Figure 4.

All traits reflecting the cheese-making efficiency of milk of PF cows, with the exception of REC_{FAT}, also improved as the trial and lactation advanced. Curd yield ($\%CY_{CURD}$) and recovery of milk solids in the curd (REC_{SOLIDS}) increased linearly from June to October, whereas the solids yield ($\%CY_{SOLIDS}$) increased in a quadratic trend, reaching maximum values in the samples taken in October. When moved to alpine pasture, the transhumant cows maintained the same $\%CY_{CURD}$ pattern as the PF cows, but $\%CY_{SOLIDS}$ was greater in August, and REC_{SOLIDS} greater in August and September ($P < 0.05$), returning to the same level as the PF cows after returning to the PF.

Daily curd production (dCY_{CURD}, Figure 4) decreased linearly in the PF cows due to a combination of the strong linear decrease in MY and the slight linear increase in $\%CY_{CURD}$. There was a notable decrease in dCY_{CURD} ($P < 0.01$) over the entire summer alpine pasturing period, but on return to the PF, the difference between the two treatments did not reach statistical significance.

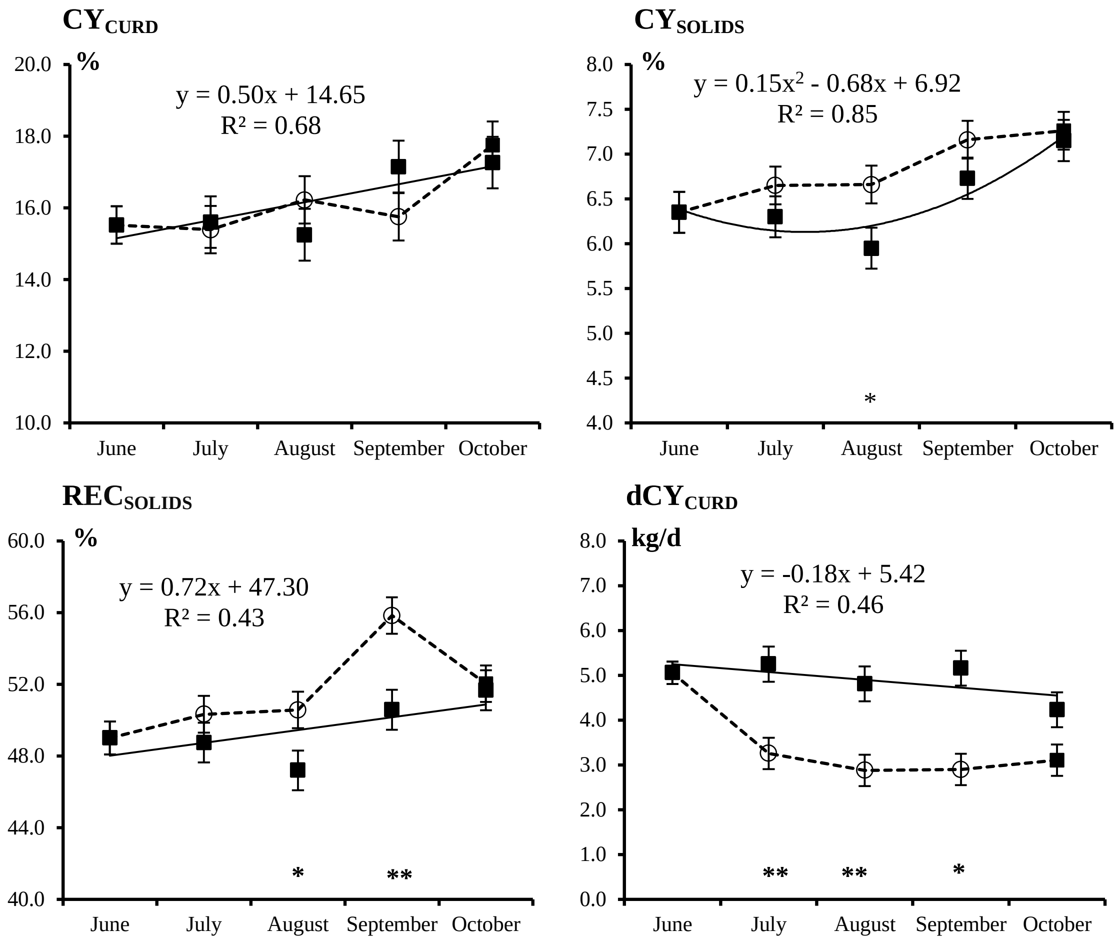

Figure 4. Least squares means and SE of the combined Treatment × Month effect for fresh cheese yield (%CY_{CURD}), total solids cheese yield (%CY_{SOLIDS}), milk solids recovered in the curd (REC_{SOLIDS}), and daily production of curd (dCY_{CURD}) of Brown Swiss cows kept on permanent farm (■ and continuous line) or moved to highland summer pasture from July to September (○ and dotted lines). Equations describe the trends of month within permanent farm (R^2 is shown). Asterisks refer to significant differences between cows moved to highland summer pasture and those kept on the permanent farm in a given month (* $P < 0.05$ or ** $P < 0.01$).

DISCUSSION

Body Condition Score and Milk Yield and Composition

The progressing months of the trial reflect the effects of advancing stage of lactation, which passed from an average of 140 DIM at the beginning to 260 DIM at the end of the trial. The pattern of change in MY, milk composition, and SCC was generally consistent with expectations for cows progressing from mid- to late lactation, namely, a decrease in MY and lactose content, and an increase in fat and protein contents and SCC (Linn, 1988; Schepers et al., 1997).

Transhumance to summer alpine pasture has notable effects on the cows' physiological, social, feeding, and nutritional status, which may affect performance and milk quality traits (Zendri et al., 2017). It involves changing from indoor barns to outdoor rearing and from a feeding strategy of mostly constant rations to grazing on pastures, frequently characterized by low productivity, limited nutritional value, sward with a high fiber content, and seasonal variation (Leiber et al., 2006; Zendri et al., 2016). In addition, the cows' activity increases through walking long distances on steep grazing areas, which may increase their energy expenditure and limit their grass intake, with negative effects on nutritional status. Transhumance to alpine pasture has been frequently associated with a reduction in feed intake (Berry et al., 2001; Leiber et al., 2004, 2006), which can reduce the energy available for metabolism. The resulting deficiency in nutrients and energy, and changes in the environment and management may explain the reduction in MY and the protein and lactose contents of the milk of the ALP cows compared with the PF cows throughout the entire summer pasturing period. The lower MY after transhumance is consistent with the findings of several studies, which report decreases in magnitudes ranging from 10 to over 40% (Leiber et al., 2006; Bergamaschi et al., 2016; Zendri et al., 2016; Niero et al., 2018). Increased milk fat content associated with the transfer of lactating cows to summer alpine pasture has also been reported by Leiber et al. (2006), Bergamaschi et al. (2016), and Niero et al. (2018), although Bugaud et al. (2001) and Zendri et al. (2016) did not find this to be the case. The increase in milk fat content has been attributed to greater body fat mobilization, which characterizes cows kept on alpine pastures compared with cows on permanent farms (Leiber et al., 2006). In the present study, we found different patterns of change in BCS in PF and ALP cows, the latter having lower scores both during alpine pasturing and after returning to the PF compared with the former. This is consistent with the findings of Leiber et al. (2004, 2005), who reported greater body fat mobilization and increased blood plasma levels of β-hydroxybutyrate and non-esterified fatty acids in cows kept in alpine conditions.

Alpine pasturing of cows has been frequently reported as having a negative effect on milk protein contents (Zemp et al., 1989; Christen et al., 1996; Romanzin et al., 2013), presumably due to hypoxia and a deficient nutrient supply (Leiber et al., 2005), despite the increasing practice of supplementing the cows' diet with concentrates (Romanzin et al., 2018). A decrease in lactose content after transhumance to summer alpine pasture has also been reported by Romanzin et al. (2013), Bergamaschi et al. (2016), and Zendri et al. (2016), although other studies found no change (Leiber et al., 2006; Niero et al., 2018).

Milk Coagulation Properties, Curd Firming, and Syneresis

In this study, the MCP parameters were on average comparable to those reported by Cecchinato et al. (2013) and Stocco et al. (2017) for the milk of Brown Swiss cows in mid and late lactation kept on mountain lowland farms. Rennet coagulation time, curd-firming time, and curd firmness of the milk from PF cows varied with month of trial (and days in milk) in a quadratic pattern: RCT and k_{20} remained stable until August, when the average DIM of cows approached 200 days, and decreased thereafter; whereas curd firmness after 30 and 45 min (a_{30} and a_{45}, respectively) increased in the last month of trial. Bittante et al. (2015) also reported a quadratic pattern of change in RCT, curd-firming time, and curd firmness with advancing stage of lactation: Milk coagulation properties decreased in the first half of lactation and progressively recovered thereafter, confirming the results of Malchiodi et al. (2014). Our results also agree with the findings of Stocco et al. (2017), who found an improvement in k_{20} and a_{30} in the second half of lactation. The pattern of change in the curd firmness model parameters of milk from PF cows as the trial progressed and DIM increased was consistent with the results of Bittante et al. (2015), confirming an improvement in milk coagulation ability in the last stage of lactation.

Alpine pasturing of dairy cows has been reported to impair the rennet coagulation time of the milk (Bovolenta et al., 1998; Leiber et al., 2005). An increase in the RCT of milk from these cows compared with cows kept in lowland barns was reported by Niero et al. (2018), who did not however observe differences in curd firmness between the treatments of cows. Leiber et al. (2006) reported impaired RCT and k_{20} in milk from cows grazed at high altitude and attributed this decrease in rennet coagulation properties mainly to the lower protein content of their milk. It is worth noting that studies on summer transhumance frequently compare lowland and highland conditions using data from the same group of cows before and during summer pasture, an experimental design that confounds the effects of changing the environment/feeding with those of advancing lactation/pregnancy. As the cows of the two treatments in our study were from the same herd, were kept in the same conditions until June, and then again in October, and had similar parities and DIM, the only difference between them was that one spent the period July–September on the ALP while the other remained on the PF. Summer pasturing of the cows, despite reducing the milk protein content, did not alter the milk coagulation properties and curd-firming process, so that the patterns of these during the entire period on the alpine pasture and after returning to the PF in the autumn were the same as those of the PF cows. Our results are partly consistent with the findings of Zendri et al. (2017), who reported that moving cows to summer pasture did not affect coagulation time and only slightly influenced the curd-firming process. Bovolenta et al. (2009) suggest that feeding concentrates to cows on mountain pasture would improve milk coagulation properties. It is therefore possible that inconsistent results concerning the effects of summer alpine pasturing on MCP may be partly due to differences in concentrate supplementation, which may mitigate the potentially detrimental effects of summer alpine pasturing on MCP.

Cheese Yield and Nutrient Recovery in Curd

The average percentage fresh cheese yield (%CY_{CURD}) found in this study is comparable to that reported by Cipolat-Gotet et al. (2013) using the same model-cheese manufacturing process and milk from Brown Swiss cows in mid- and late lactation reared in mountain lowland farms. All %CY traits of milk from PF cows increased with the progressing month of the trial and DIM, with patterns of variation consistent with those reported by Cipolat-Gotet et al. (2013) and Stocco et al. (2018), who observed a progressive increase in %CY_{CURD}, %CY_{SOLIDS} and REC_{SOLIDS} from the second month to the end of lactation. This pattern is consistent with the observed improvement in milk nutrients contents.

The authors are unaware of any study comparing the cheese yield of milk obtained during summer pasturing with that of milk obtained in the lowlands. The average %CY_{CURD} values found in this study using the individual model cheese-making procedure were slightly higher than those obtained by Bergamaschi et al. (2016) in cheeses made from milk from the same ALP as this study but using an artisanal cheese-making procedure. The values for milk nutrient recovery in the curd found here are also in agreement with those reported by Bergamaschi et al. (2016).

In the current study, the summer pasturing of cows did not alter the %CY_{CURD}, and ALP and PF cows had similar fresh cheese yields during the entire period of alpine pasturing, although the cows at pasture had somewhat greater $\%CY_{SOLIDS}$ and REC_{SOLIDS} than those kept on the permanent farm. The differences between the treatments remained more or less the same after the cows returned from the alpine pasture to the PF in autumn. In a survey of 15 temporary summer farms, Zendri et al. (2017) predicted cheese yield traits on the basis of Fourier-transform infrared spectrometry of milk samples. They reported a negative effect of the transhumance on $\%CY_{CURD}$ in the first month at pasture, but thereafter a progressive increase in fresh cheese yield, coinciding with the advancing of DIM and the cows adapting to the conditions after the initial stress. However, without a lowland control group, the study's experimental design confounds the effects of DIM with the effects of transhumance.

Daily fresh cheese yield decreased linearly in PF cows with advancing DIM, a trend that was expected and consistent with the findings of Cipolat-Gotet et al. (2013) on lowland farms. The differences between animals of the two treatments in dCY_{CURD} reflect differences in MY and $\%CY_{CURD}$ between the two groups: During the months at pasture, ALP cows produced nearly 2 kg/d less fresh cheese than PF cows, a gap that narrowed only partially after their return to the permanent farm in autumn.

CONCLUSIONS

This study presents new information on the effects that summer transhumance of dairy cows to alpine pastures exerts on the yield, composition, and cheese-making properties of milk, by comparing the performance of two homogeneous groups of cows from the same permanent farm before, during, and after the transfer of one group to summer highland pastures.

The results show that transhumant cows had lower body condition scores, and reduced milk yield and milk protein content, although milk traits were partially recovered after their return to the lowland permanent farm. From this, we can confirm that transhumance to alpine pastures may affect productive functions and body fat reserves, even when cows are given concentrate supplements. On the other hand, the cheese-making attributes of milk were not affected by summer transhumance: Milk coagulation properties, curd-firming parameters, and cheese yield were nearly identical for cows transferred to alpine pasture and those that remained on the lowland permanent farm. A consequence of the adverse impact of alpine pasturing on milk yield was the substantially lower daily cheese yield from transhumant cows than from cows remaining on the permanent farm, although this effect tended to narrow after the cows returned to the permanent farm. The production losses resulting from alpine grazing need to be compensated for by creative marketing of alpine products and adequate remuneration of the environmental, recreational, social, and cultural services provided by the transhumance of dairy cows to mountain pastures.

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CHAPTER 3

Milk coagulation traits and cheese yields of purebred Holsteins and four generations of three-breed rotational crossbred cows from Viking Red, Montbéliarde, and Holstein bulls

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ABSTRACT

Crossbreeding is a strategy to counter the declining fertility, resilience and longevity of purebred Holstein (**Ho**) cows. However, little is known of the effects of long-term systematic rotational crossbreeding on milk technological properties and cheese yield. In this study we compared the milk composition, coagulation properties (**MCP**) and individual cheese yields (**CY**) of 468 purebred Ho and 648 crossbred (**CR**) cows obtained from two 3-breed rotational crossbreeding systems using Viking Red (**VR**), Montbéliarde (**Mo**) and Ho sires over 4 generations. Individual milk samples were collected once from 1116 primiparous and multiparous cows kept in two dairy herds, raised for the production of Grana Padano (high milk yield, total mixed ration based on corn silage) and Parmigiano Reggiano cheese (moderate milk yield, only dry feeds). In both herds, a 3-breed rotational mating system was used, in which Ho cows were first inseminated with VR, whereas Mo and Ho semen was used in the subsequent generations. In one herd, the sequence Mo-VR-Ho was also used. Individual milk samples were analyzed for milk composition, single-point MCP, and parameters for modeling curd-firming over time, whereas CY and milk nutrient recovery in the curd (**REC**) were assessed through a laboratory cheese-making procedure. Compared with Ho, CR cows produced 5.8% less milk, with comparable fat but greater protein and casein contents, and lower lactose contents and somatic cell scores (**SCS**). Milk from CR cows reached a curd firmness of 20 mm more quickly, and exhibited greater curd firmness at 30, 45 and 60 min from rennet addition. Ho and CR cows yielded milk with very similar CY and REC traits. The milk fat content, SCS, curd firmness traits and CY of CR cows relative to the Ho cows differed in the two herds, and the favorable effects on the CR cows were more evident in the herd with the greatest milk yield and the worst MCP traits. Crossbred cows of the four generations performed similarly, with the exception of the better MCP of the milk from F1 CR cows. The two rotational systems using different sire-breed sequences also performed similarly. In summary, both rotational crossbreeding programs exhibited some advantage over the Ho purebred breeding system in terms of milk composition and MCP, but not CY. Future research is needed to investigate the interactions between crossbreeding schemes and dairy systems.

Keywords: dairy cow, crossbreeding, milk quality, cheese-making efficiency

INTRODUCTION

There has been growing interest in crossbreeding for commercial dairy production, due to the deterioration in fertility, health, and longevity, the increasing rate of inbreeding in major dairy breeds (Buckley et al., 2014), and changes in milk pricing towards a focus on fat and protein contents (Weigel and Barlass, 2003; Shonka-Martin et al., 2019b).

Holstein (**Ho**) is the major dairy breed worldwide as a result of its ability to produce high volume of milk (Blöttner et al., 2011). However, greatly increased milk production and unfavorable phenotypic correlations led to cows being less fertile, more prone to health problems, and less enduring (Berry, 2018), despite the recent emphasis on improving fertility and health traits (VanRaden, 2018). Interest has therefore grown in crossing Ho cows with other breeds to compensate for these adverse effects. A three-breed rotational crossbreeding program is currently gaining attention, particularly in intensive dairy systems with little opportunity for grazing (Dechow and Hansen, 2017). This type of program often involves the Montèliarde (**Mo**) and Viking Red (**VR**) breeds, the latter including the Swedish Red, Finnish Ayrshire, and Danish

Red subpopulations (Shonka-Martin et al., 2019b), whereas the Jersey breed seems more suited for crossbreeding programs aimed to grazing systems (Vance et al., 2012).

Crossbreeding is now widespread in many different countries. For example, in 2015 crossbred cows accounted for around 50% of dairy cattle born in New Zealand (LIC and Dairy NZ, 2018). There has been a 9-fold increase in crossbred cows over the last decade in USA, (Hazel et al., 2017b), while in Sweden they now account for 8% of the overall cattle population, and in Denmark 12% (Clasen et al., 2019).

Most of the previous studies on crossbreeding in dairy cattle have focused on fertility (Malchiodi et al., 2014a; Hazel et al., 2017a), survivability (Hazel et al., 2014), body traits (Blöttner et al., 2011; Saha et al., 2018), and milk yield and quality (Hazel et al., 2017b).

Cheese is consistently the major dairy product worldwide, with more than 36% of milk processed into cheese products, a proportion that has increased by 23% in the last decade (International Dairy Federation, 2016). Cheese production is also the main use of milk in many European countries, such as Italy, where 75% of milk is destined for cheese production, and 55% of the total milk supply is processed into Protected Designation of Origin (**PDO**) cheeses. The technological and cheese-making properties of milk are therefore increasingly critical traits for the dairy industry, as milk quality is related to optimal cheese yield and quality (Malacarne et al., 2006, Skeie, 2007). However, the technological properties of milk from crossbred cows have so far received little attention. In one of few studies, Malchiodi et al. (2014b) compared the milk coagulation properties (**MCP**) of purebred Ho and two generations of crossbred cows obtained from Ho, VR, Mo and Brown Swiss breeds in one dairy system. Cheese-making properties and milk nutrient recovery in cheese have not as yet been investigated.

Focusing on the yield, composition, coagulation properties and cheese yield (**CY**) of milk, the aims of this study were: 1) to compare purebred Ho with three generations of crossbred cows obtained from a 3-breed rotational crossbreeding system using VR, Mo and Ho sires, and 2) to compare four different generations of crossbred cows obtained from two 3-breed rotational crossbreeding systems differing in their sire-breed sequences (VR-Mo-Ho and Mo-VR-Ho, respectively).

MATERIALS AND METHODS

Herds, Animals and Crossbreeding Programs

The study involved 1116 dairy cows from two dairy herds located in northern Italy. The crossbred cows were obtained from a 3-breed rotational crossbreeding system named ProCross, which is managed by Coopex Montbéliarde (Roulans, France) and Viking Genetics (Randers, Denmark). Both farms (Table 1) used the VR-Mo-Ho sequence, i.e. VR semen was used on Ho purebred cows to produce the 1st generation (F1) of VR×Ho crossbred cows, Mo semen was used on the F1 cows to produce the 2nd generation (F2) of Mo×(VR×Ho) crossbred cows, and Ho semen was used on the F2 cows to produce the 3rd generation (F3) of Ho×[Mo×(VR×Ho)] crossbred cows; the sequence then started again with VR and so on. In herd B, however, a second crossbreeding program was also used, in which VR and Mo were reversed in the sequence (Mo-VR-Ho), i.e. Mo semen was used on the Ho cows to produce the F1 Mo×Ho, which were inseminated with VR semen to produce the F2 crossbred cows, which were inseminated with Ho semen to produce the F3 crossbred cows; the sequence was then repeated using Mo sires to obtain the 4th generation (F4) crossbred cows, and so on (Table 1). At least one third of the cows in both herds were maintained as purebred Holsteins. As both farms were increasing the number of crossbred cows produced from purebred Ho, new F1 cows were obtained every year, so that cows of every generation and parity were present on the farms at the same time, and only the F4 cows were of on average younger age than the other crossbreds.

Table 1. Rotational crossbreeding sequences and numbers of cows sampled for milk yield, composition, and technological properties for dataset 1 (all purebred Ho and three generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herd A and herd B) and for dataset 2 (four generations of two 3-breed rotational [VR-Mo-Ho and Mo-VR-Ho] crossbred cows of herd B only). (Ho = Holstein, VR = Viking Red, Mo = Montbéliarde).

Breed combinations:	Cow number:		Dataset:	
	Herd A	Herd B	1	2
Purebred Ho	195	273	468	
3-breed rotational VR-Mo-Ho				
VR×Ho (F1)	35	47	82	47
Mo×(VR×Ho) (F2)	42	53	95	53
Ho×[Mo×(VR×Ho)] (F3)	48	137	185	137
VR×{Ho×[Mo×(VR×Ho)]} (F4)		60		60
3-breed rotational Mo-VR-Ho				
Mo×Ho (F1)		105		105
VR×(Mo×Ho) (F2)		71		71
Ho×[VR×(Mo×Ho)] (F3)		39		39
Mo×{Ho×[VR×(Mo×Ho)]} (F4)		11		11
Total	320	796	830	523

Only semen from bulls registered in the herd books was used for artificial insemination. Overall, 133 Ho bulls were used as sires of purebred and F3 crossbred cows (on average 5.2 cows/sire), while 19 VR bulls (on average 11.2 cows/sire) and 25 Mo bulls (on average 8.4 cows/sire) were used as sires of the F1, F2 and F4 crossbred cows.

Both herds, which were representative of the two most important dairy systems in Italy, kept cows in free stalls with cubicles, and fed total mixed ration. Herd A was located on the Lombardia plains (province of Brescia), in the Grana Padano PDO hard cheese production area, and its rations were accordingly based mainly on corn silage, sorghum silage, and concentrates. Herd B was located in the Emilia-Romagna region (province of Modena), in the Parmigiano

Reggiano PDO hard cheese production area. As the regulations governing production of this cheese forbid the use of silages, cows kept in the herd B were fed a ration based on dry roughage, mainly alfalfa and meadow hay, and concentrates; its production level was therefore expected to be lower than that of herd A. Within each herd purebred Ho and crossbred cows were reared and milked together and were fed the same diets.

Milk Sampling and Milk Yield and Composition

Individual milk samples (100 mL) were collected from all cows in each herd in one sampling session per each herd during a winter evening milking. After collection, the milk samples (without preservative) were immediately stored in a refrigerator at -20^{o} C and transferred to the Milk Quality Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and the Environment (DAFNAE) of the University of Padova (Italy) for analysis.

Milk yield and milk fat and protein contents of milk were obtained from the official Italian recording system (Milkoscan FT6000 infrared analyzer, Foss Electric A/S, Hillerød, Denmark). Casein, lactose and total solids contents were measured with a Milkoscan FT2 infrared analyser (Foss Electric A/S, Hillerød, Denmark). Milk pH was measured using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Somatic cell count was obtained using a Fossomatic Minor FC counter (Foss Electric A/S) and was then converted to SCS by using the log transformation proposed by Ali and Shook (1980).

Milk Coagulation and Curd Firmness Properties

Curd firmness was assessed in duplicate on each sample from each cow every 15 s for 60 min (240 measures from each milk sample and each replicate) using a lactodynamograph

(Formagraph; Foss Electric, A/S, Hillerød, Denmark) and according to the procedure described by Stocco et al., (2017). Frozen milk was thawed overnight in a refrigerator at 4^{o}C, and 10 mL of milk was heated to 35^o C then mixed with 200 μL of rennet solution (Hansen Standard 215 with 80 ± 5 % chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2% (wt/vol) with distilled water. Traditional single point MCP traits were obtained from the lactodynamograph, namely: rennet coagulation time (RCT, min), defined as the time from rennet addition to milk gelation; curd firming time (k_{20}, min), defined as the time taken from gelation to reach a curd firmness of 20 mm; and curd firmness 30, 45 and 60 min after rennet addition (a_{30}, a_{45} and a_{60}, respectively, mm).

In addition, all 240 curd firmness records from each milk sample and replicate were modeled using the equation proposed by Bittante et al. (2013) to provide estimates of the following individual curd firming and syneresis parameters: rennet coagulation time estimated from the individual curd firming equations (RCT_{eq}, min), the curd firming instant rate constant ($k_{CF,}$ % × min^{-1}), the curd syneresis instant rate constant ($k_{SR,}$ % × min^{-1}), maximum curd firmness value (CF_{max}, mm) and the time to CF_{max} (t_{max}, min).

Cheese-Making Procedure and Cheese Yield Traits

We used the 9-MilCA method proposed by Cipolat-Gotet et al (2016) to assess in duplicate on each sample cheese yield and milk nutrient recovery in the curd. Each milk sample (9 mL) was poured into a glass tube, placed in a sample rack, heated at 35^o C for 15 min, then gently mixed with 0.2 mL rennet solution (Hansen Standard 215, with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland), diluted to 1.2% (wt/vol) with distilled water. After 30 min at 35^o C, samples were manually cut with a stainless steel spatula then heated at 55^{o} C for 30 min (curd cooking phase). At 15 min (the middle of this phase), each sample was manually cut. At the end of cooking, each glass tube was removed from the sample rack and the curd was separated from the whey for 30 min at room temperature, then lightly pressed with the same spatula as used for cutting the curd to expel the whey. The resulting whey and curd were weighed using precision scales. The whey drained from the curd was analyzed for fat, protein, and total solids contents using an infrared spectrophotometer (Milkoscan FT2, Foss Electric). We determined three CY traits (CY_{CURD}, CY_{SOLIDS} and CY_{WATER}) expressing, respectively, the weight of the fresh curd, curd solids and curd water as percentage of the milk processed, and two milk nutrient recovery in the curd (REC) traits ($REC_{PROTEIN}$ and $REC_{FAT,}$ %) from the differences in weight and composition between the milk and the whey (Cipolat-Gotet et al., 2016).

Editing Procedures and Statistical Analysis

Replicated measures of the same trait were averaged before statistical analysis to obtain one record per cow for all traits of concern. All records were classified for parity (**PAR**, 3 classes: 1, 2, and ≥3), DIM (5 classes: <60 d; 60 to 120 d; 121 to 180 d; 181 to 240 d; >240 d), **HERD** (2 classes: herd A and herd B), date of sample analysis (**DATE**, 39 classes) and breed combinations (**BREED**, 9 classes: purebred Ho; two F1: VR×Ho and Mo×Ho; two F2: Mo×(VR×Ho) and VR×(Mo×Ho); two F3: Ho×[Mo×(VR×Ho)] and Ho×[VR×(Mo×Ho)]; and two F4: VR×{Ho×[Mo×(VR×Ho)]} and Mo×{Ho×[VR×(Mo×Ho)]} crossbred cows). The numbers of cows per breed combination are given in Table 1.

We compiled two different datasets from the data, the descriptive statistics of which are given in Supplemental Table S1 (see appendix).

Dataset 1

This dataset included the data from all purebred Ho, and F1, F2 and F3 crossbred combinations from the rotational sequence VR-Mo-Ho used in both herds (830 cows; Table 1). We used the SAS generalized linear model procedure to analyze the milk yield and milk fat and protein contents, including the fixed effects of HERD, PAR, DIM, BREED, and the HERD × BREED interaction. We analyzed all the other traits using a mixed model procedure, which included the random effect of DATE nested within HERD, and the fixed effects of HERD, PAR, DIM, BREED, and the HERD × BREED interaction. Orthogonal contrasts were estimated between the least squares means (**LSM**) of the breed combinations to compare the performance of purebred Ho with the average performance of crossbred cows, and between the LSM of the HERD × BREED interaction to assess possible differences between the two herds in the Ho *vs* crossbred cows comparison.

Dataset 2

This dataset included the data from all the F1, F2, F3 and F4 crossbred cows in herd B produced from the two different rotational sequences (523 cows; Table 1). We used the SAS generalized linear model procedure to analyze the milk yield and milk fat and protein contents, including the fixed effects of PAR, DIM, and BREED. We analyzed all the other traits using a mixed model procedure, which included the random effect of DATE, and the fixed effects of PAR, DIM, and BREED. Orthogonal contrasts were estimated between the LSM of the breed combinations in order to 1) compare the performances of the two rotational crossbreeding sequences, VR-Mo-Ho *vs* Mo-VR-Ho; 2) compare the performance of the different generations [F1 *vs* (F2 + F3 + F4); F2 *vs* (F3 + F4); F3 *vs* F4]; and 3) compare the performance of the crosses obtained from the two rotational sequences within generation (VR×Ho *vs* Mo×Ho within F1;

Mo×(VR×Ho) *vs* VR×(Mo×Ho) within F2; Ho×[Mo×(VR×Ho)] *vs* Ho×[VR×(Mo×Ho)] within F3; VR×{Ho×[Mo×(VR×Ho)]} *vs* Mo×{Ho×[VR×(Mo×Ho)]} within F4).

RESULTS

Comparison between Purebred Holstein and Crossbred Cows (dataset 1)

Milk Yield and Composition

The herds differed significantly with regard to milk yield and composition (Table 2), the cows from herd A (Grana Padano dairy system using silages) producing nearly 4 kg/d more milk (+ 12%) than herd B (Parmigiano-Reggiano dairy system using only dry feeds), but with a lower protein content (-3%). Breed combination also significantly affected the milk yield and composition traits. On average, purebred Ho yielded nearly 35 kg/d of milk, 5.8% more than the crossbred cows, which yielded nearly 33 kg/d of milk ($P < 0.01$). Milk from the crossbred cows had a fat content comparable to the milk from Ho cows, but greater protein and casein contents, and lower lactose contents and SCS ($P < 0.01$). The fat and lactose contents, and SCS of the crossbred cows relative to the purebred Ho cows differed in the two herds ($P < 0.05$): the milk from crossbred cows had a nearly 4% greater fat content than the milk from purebred Ho in herd A, but they were nearly the same in the herd B. Similarly, SCS was 20% lower in milk from crossbred cows than in milk from purebred Ho cows in herd A, but they were nearly the same in herd B (Figure 1).

Table 2. Comparison between purebred Holsteins and 3 generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herds A and B (dataset 1): LSM and SEM for milk yield (MY), milk nutrient contents and somatic cell score (SCS) (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows, no.	MY, kg/d	Fat, %	Protein, %	Casein, %	Lactose, %	SCS[1]
Herds[1]:							
A	320	35.4[a]	3.94	3.63[b]	2.87[b]	5.06	2.75
B	510	31.4[b]	3.81	3.73[a]	3.02[a]	5.03	2.59
SEM	-	0.5	0.05	0.02	0.02	0.01	0.12
Breed combinations:							
Purebred Ho	468	34.9	3.80	3.63	2.89	5.08	2.93
VR×Ho (F1)	82	33.3	4.02	3.74	3.00	5.01	2.74
Mo×(VR×Ho) (F2)	95	32.1	3.77	3.63	2.92	5.03	2.52
Ho×[Mox(VR×Ho)] (F3)	185	33.5	3.91	3.72	2.96	5.07	2.48
SEM	-	0.7	0.07	0.03	0.02	0.02	0.14
Breed effect (*P* value)	-	0.01	0.13	0.004	0.002	0.0007	0.008
Contrasts, *P* value:							
Ho vs crossbreds (F1+F2+F3)	-	0.002	0.14	0.01	0.0008	0.0002	0.003
(Ho vs crossbreds) × herd	-	0.37	0.05	0.21	0.38	0.01	0.03

[1] LSM across columns with different superscripts are significantly different ($P < 0.05$)
[2]SCS = 3 + log2 (SCC/100,000).

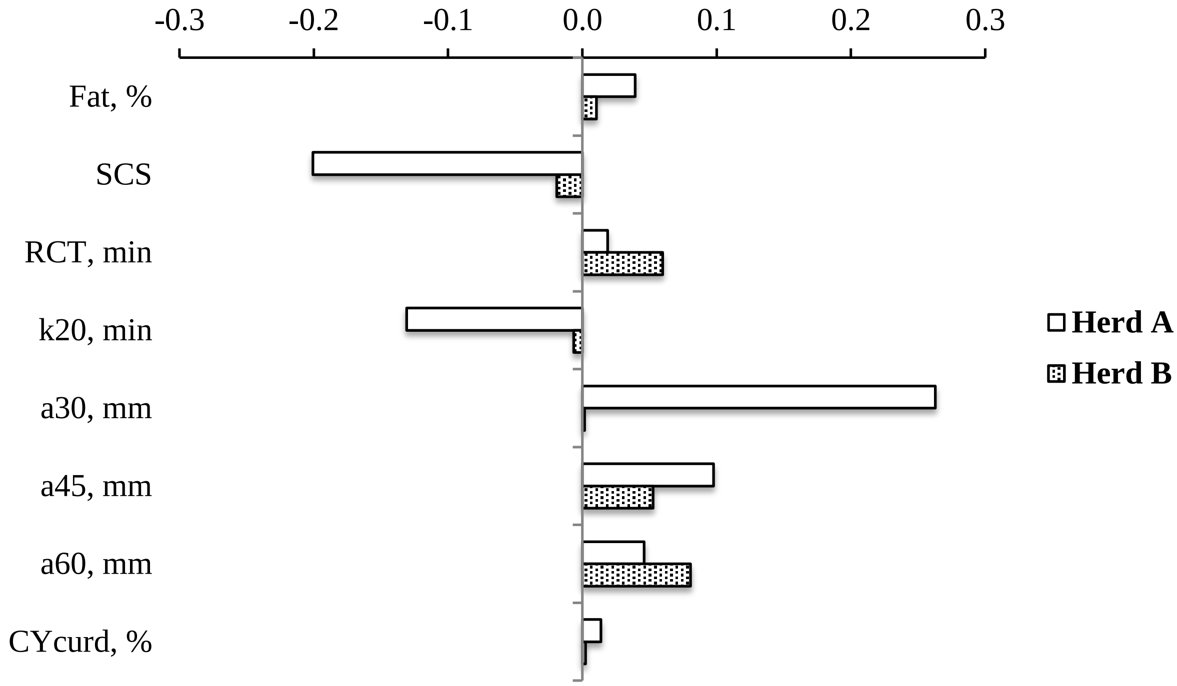

Figure 1. LSM of crossbred cows expressed as the deviation from the LSM of Ho cows shown separately for the two herds for traits with a significant (P < 0.05) interaction between herd and breed (data set 1). (RCT = measured rennet coagulation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30}, a_{45}, a_{60} = curd firmness after 30, 45 and 60 min from rennet addition, respectively; CYcurd = fresh cheese yield).

Single Point MCP and Curd Firming (CF_t) Equation Parameters

The LSM of single point MCP across herds and breeds are reported in Table 3. On average, the samples coagulated about 24 min after rennet addition (RCT), and reached a curd firmness of 20 mm (k_{20}) after nearly 6 min. Average curd firmness at 30 min was around 31 mm, and increased to 36 mm at 45 min from rennet addition. The coagulation properties of milk were very different in the two herds, the milk from herd B being superior to the milk from herd A for all traits (P < 0.05). Breed also affected all single point MCP, with the only exception of RCT. The incidence of non-coagulating samples was comparable in purebred Ho and crossbred cows, and close to 20%, 6% and 4% at 30, 45 and 60 min from rennet addition, respectively (data not shown). Milk from the crossbred cows had similar RCT to the milk from purebred Ho, but took less time to reach a curd firmness of 20 mm (k_{20}), and had a greater curd firmness at 30, 45 and 60 min from rennet addition. The MCP of purebred Ho cows relative to the crossbred cows also differed in the two herds: the milk from crossbred and Ho cows in herd A had similar RCT, whereas in herd B the milk from Ho cows had a slightly shorter RCT. Moreover, k_{20}, and a_{30} were much better in milk from crossbred than from purebred Ho cows in herd A, but were nearly identical in purebred and crossbred cows in herd B (Figure 1).

Table 3. Comparison between purebred Holsteins and 3 generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herds A and B (dataset 1): LSM and SEM for single point milk coagulation properties (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows no.	Milk coagulation properties[1]				
		RCT, min	k_{20}, min	a_{30}, mm	a_{45}, mm	a_{60}, mm
Herds[2]:						
A	320	31.7[a]	8.5[a]	19.9[b]	26.7[b]	25.9[b]
B	508	19.9[b]	4.7[b]	35.8[a]	41.8[a]	39.6[a]
SEM	-	0.7	0.3	1.5	1.4	1.6
Breed combinations:						
Purebred Ho	467	25.2	7.1	26.2	32.5	31.2
VR×Ho (F1)	82	27.1	6.6	31.9	33.8	31.7
Mo×(VR×Ho) (F2)	95	26.3	6.8	24.1	33.7	33.1
Ho×[Mox(VR×Ho)] (F3)	184	24.6	5.9	29.2	37.0	34.8
SEM	-	0.8	0.3	1.8	1.4	1.4
Breed effect (*P v*alue)	-	0.13	0.002	0.02	0.002	0.0005
Contrasts, *P* value:						
Ho vs crossbreds (F1+F2+F3)	-	0.17	0.009	0.10	0.01	0.004
(Ho vs crossbreds) × herd	-	<.0001	0.03	0.07	0.02	0.02

[1]RCT = rennet coagulation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} (a_{45}, a_{60}) = curd firmness after 30 (45, 60) min from rennet addition.
[2]LSM across columns with different superscripts are significantly different ($P < 0.05$)

The LSM of the curd firming equation parameters across herds and breeds are reported in Table 4. The average coagulation time of each milk sample calculated on the basis of all 240 data points (RCT_{eq}) was 24 min. The average coagulation time of each milk sample calculated on the basis of all 240 data points (RCT_{eq}) was 24 min. The average asymptotic potential curd firmness theoretically achievable at infinite time in absence of curd syneresis (CF_P) was close to 58 mm, while the average instant rate constant of curd firming (k_{CF}) and of syneresis (k_{SR}) was 9.22 and 1.13% × min^{-1}, respectively. On average, the maximum CF value (CF_{max}) was 38.6 mm, and was achieved 47 min after rennet addition (t_{max}).

Table 4. Comparison between purebred Holsteins and 3 generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herds A and B (dataset 1): LSM and SEM for curd firming (CF_t) equation parameters (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows no.	CF_t equation parameters[1]					
		RCT_{eq}, min	k_{CF}, % × min^{-1}	k_{SR}, % × min^{-1}	CF_p, mm	CF_{max}, mm	t_{max}, min
Herds[2]:							
A	320	31.9[a]	9.8	0.7[b]	39.0[b]	28.5[b]	52.1[a]
B	508	20.4[b]	9.7	1.2[a]	66.6[a]	45.3[a]	44.8[b]
SEM	-	0.7	0.5	0.1	2.1	1.3	1.0
Breed combinations:							
Purebred Ho	467	25.6	9.9	1.0	51.0	35.6	47.7
VR×Ho (F1)	82	27.3	10.1	0.9	52.1	36.3	48.5
Mo×(VR×Ho) (F2)	95	26.6	9.3	0.9	51.6	36.1	49.4
Ho×[Mox(VR×Ho)] (F3)	184	25.1	9.7	1.0	56.4	39.5	48.2
SEM	-	0.8	0.4	0.1	1.9	1.2	1.0
Breed effect (*P* value)	-	0.21	0.26	0.15	0.003	0.002	0.38
Contrasts, *P* value:							
Ho vs crossbreds (F1+F2+F3)	-	0.28	0.28	0.04	0.04	0.04	0.13
(Ho vs crossbreds) × herd	-	< 0.0001	0.47	<0.0001	<0.0001	0.0002	<0.0001

[1]RCT_{eq} = RCT estimated according to curd firm change over time modeling (CF_t); k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_P = asymptotic potential curd firmness; CF_{max} = maximum curd firmness; t_{max} = time to reach CF_{max}.
[2] LSM across columns with different superscripts are significantly different ($P < 0.05$)

There were significant differences between the herds with respect to most of the CF_t equation parameters, suggesting large differences in the coagulation times and curd firming patterns of milk from the two herds. CF_t equation parameters were also affected by breed, as milk from the crossbred cows had greater potential and maximum curd firmness, and a slower syneresis rate than milk from the purebred Ho cows. Although the comparisons between purebred Ho and crossbred cows with respect to the CF_t equation parameters mostly differed between the two herds (P < 0.01), the magnitude of these differences was smaller than that between the two herds. Indeed, as Figure 2 shows, the milk produced by herd B was much more suited to cheese production than the milk from herd A. In both herds, the milk from the crossbred cows was similar to the milk from the Ho cows with regard to coagulation and the first part of the curd firming process (RCT_{eq} and k_{CF} not significantly different), but was clearly superior thereafter, with greater CF_P and CF_{max}, and a much lesser decrease due to syneresis (k_{SR}). Moreover, there is a clear significant interaction between herd and breed combination as the superiority of the milk from crossbred cows is more evident in the case of herd B than of herd A (Figure 2).

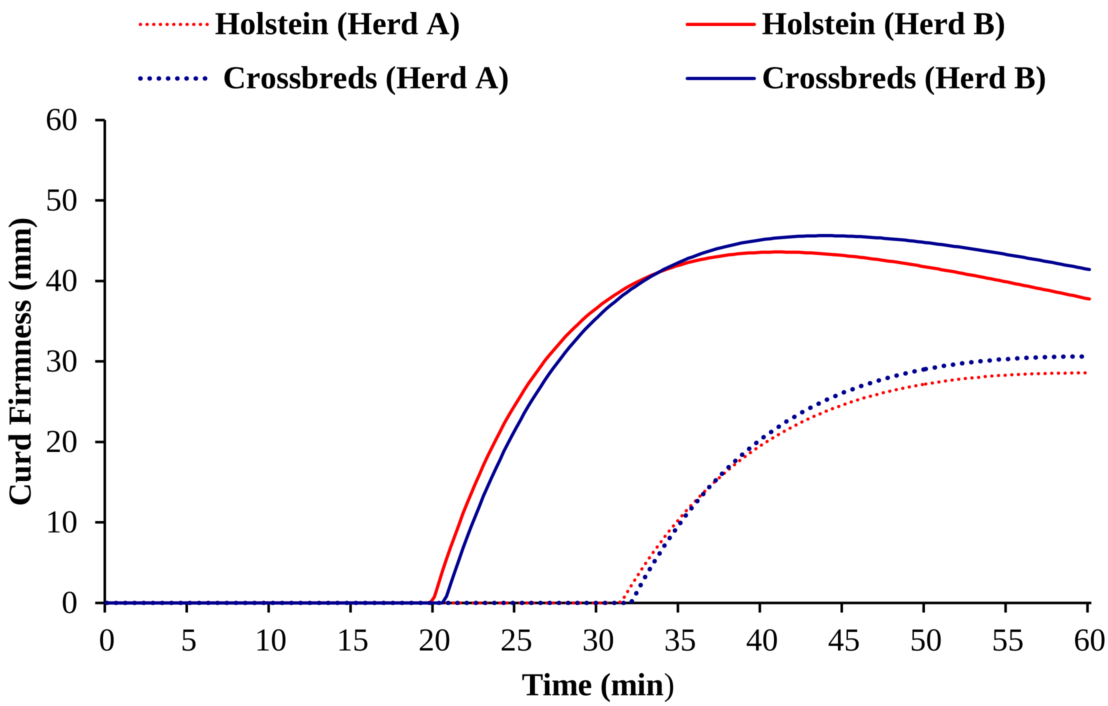

Figure 2. Pattern of curd firming after rennet addition (CF_t equation) of milk samples from Holstein and 3-breed rotational crossbred cows (average of F1, F2 and F3 breed combinations) kept in herds A and B (Data set 1). Color version is available online.

Cheese Yield and Milk Nutrient Recovery in the Curd

The LSM for cheese yield and milk nutrient recovery in the curd across herds and breeds are given in Table 5. On average, the milk yielded 170 g fresh curd/kg, (48 g of solids, 122 g of retained water), with a recovery in the curd of nearly 80% of the milk protein and 57% of the fat contents. The differences in CY between the herds ($P < 0.05$) were entirely due to the different water contents entrapped in the curd, as solid CY was nearly identical in the two herds. Milk protein recovered in the curd was also similar in the two herds, but we found differences in REC_{FAT}. Both purebred Ho and crossbred cows yielded milk with very similar CY coefficients, and REC traits. Comparison of CY in purebred Ho and crossbred cows revealed significant differences between the two herds ($P = 0.04$), as in herd A the CY of crossbred cows was nearly

1.5% greater than that of purebred Ho, whereas in herd B the CY of purebred Ho and crossbred cows were nearly identical (Figure 1).

Table 5. Comparison between purebred Holsteins and 3 generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herds A and B (dataset 1): LSM and SEM for cheese yield (CY) and milk nutrient recovery in curd (REC) (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows no.	CY[1], %			REC[2], %	
		Curd	Solids	Water	Protein	Fat
Farms[3]:						
A	320	17.56[a]	4.86	12.70[a]	79.84	58.43[a]
B	510	16.68[b]	4.83	11.83[b]	80.18	56.52[b]
SEM	-	0.21	0.07	0.16	0.17	1.00
Breed combinations:						
Purebred Ho	468	17.02	4.80	12.22	79.92	57.37
VR×Ho (F1)	82	17.30	4.99	12.33	80.02	57.25
Mo×(VR×Ho) (F2)	95	16.83	4.67	12.12	80.01	57.11
Ho×[Mox(VR×Ho)] (F3)	185	17.33	4.91	12.39	80.08	58.17
SEM	-	0.23	0.08	0.19	0.22	1.23
Breed effect (*P* value)	-	0.30	0.02	0.73	0.91	0.91
Contrasts, *P* value:						
Ho vs crossbreds (F1+F2+F3)	-	0.44	0.28	0.72	0.53	0.89
(Ho vs crossbreds) × herd	-	0.04	0.29	0.05	0.10	0.07

[1]CY_{CURD} = fresh cheese yield; CY_{SOLIDS} = total solids cheese yield; CY_{WATER} = water entrapped in the curd.
[2]$REC_{PROTEIN}$ = milk protein retained in the curd; REC_{FAT} = milk fat retained in the curd.
[3]LSM across columns with different superscripts are significantly different ($P < 0.05$)

Comparison among Crossbred Cows (data set 2)

Milk Yield and Composition

Milk yield and composition traits for the different crossbreed combinations are given in Table 6. On average, crossbred cows produced nearly 30 kg milk per day, with fat and protein contents close to 3.80 and 3.75%, respectively. The sire-breed sequence did not affect milk yield and composition, except for average milk protein and lactose contents. Indeed, starting the crossbreeding program with VR rather than Mo sires slightly improved the average milk protein contents (3.77 *vs* 3.70%) and slightly reduced average milk lactose contents (5.00 *vs* 5.05%). The performances of cows from different crossbred generations were similar in terms of milk yield and composition, with few exceptions (F1 *vs* other generations for casein content; F3 *vs* F4 for fat content). Likewise, the performances of crosses from the two rotational sequences and of the same generation were generally similar, the only difference being the protein content of milk from F4 crossbred cows.

Table 6. Comparison between 4 generations of crossbred cows from two rotational sequences (VR-Mo-Ho and Mo-VR-Ho; dataset 2, herd B): LSM and SEM for milk yield (MY), milk nutrient contents and somatic cell score (SCS) (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows no.	MY, kg/d	Fat, %	Protein, %	Casein, %	Lactose, %	SCS[1]
Breed combinations:							
Rotation VR-Mo-Ho:							
VR×Ho (F1)	47	31.1	3.95	3.80	3.11	5.01	2.68
Mo×(VR×Ho) (F2)	53	29.8	3.73	3.67	2.97	5.00	2.49
Ho×[Mo×(VR×Ho)] (F3)	137	31.2	3.77	3.73	3.00	5.04	2.50
VR×{Ho×[Mo×(VR×Ho)]} (F4)	60	29.1	4.07	3.87	3.10	4.96	2.49
Rotation Mo-VR-Ho:							
Mo×Ho (F1)	105	31.2	3.77	3.73	3.03	5.08	2.61
VR×(Mo×Ho) (F2)	71	29.3	3.86	3.76	3.05	5.03	2.76
Ho×[VR×(Mo×Ho)] (F3)	39	31.5	3.56	3.67	3.00	5.03	2.74
Mo×{Ho×[VR×(Mo×Ho)]} (F4)	11	31.0	3.90	3.66	2.94	5.04	2.30
SEM	-	1.09	0.12	0.04	0.04	0.02	0.22
Breed effect (*P v*alue)	-	0.34	0.06	0.002	0.05	0.003	0.87
Contrast, *P* value:							
VR-Mo-Ho vs Mo-VR-Ho sequence	-	0.58	0.23	0.03	0.18	0.03	0.70
F1 vs (F2+F3+F4)	-	0.32	0.59	0.21	0.04	0.21	0.55
F2 vs (F3+F4)	-	0.24	0.77	0.63	0.97	0.87	0.54
F3 vs F4	-	0.36	0.03	0.19	0.67	0.25	0.41
Within F1	-	0.43	0.15	0.57	0.76	0.96	0.39
Within F2	-	0.68	0.36	0.09	0.12	0.38	0.31
Within F3	-	0.80	0.14	0.16	0.99	0.72	0.36
Within F4	-	0.43	0.51	0.02	0.06	0.13	0.68

[1]SCS = 3 + log2 (SCC/100,000).

Single point MCP, Curd Firming Pattern and Cheese Yield

The LSM for single point MCP and CY across the different crossbreed combinations are shown in Table 7, while those for CF_t equation parameters can be found in Supplemental Table S2 (see appendix).

Whether the crossbreeding program began with VR or Mo sires did not affect the coagulation properties and the CY of milk (Table 7). However, compared with crossbred cows of the other generations, the milk from F1 crossbreds had better single point RCT, reached a curd firmness of 20 mm more quickly, and had a stronger curd firmness at 30, 45 and 60 min from rennet addition ($P < 0.05$). The superior single point MCP traits of F1 crossbred cows was due to their more favorable pattern of curd firming after rennet addition compared with crossbred cows of the other generations. Aside from F1, the cows from the other crossbred generations had comparable single point MCP traits. Likewise, the MCP traits of crosses of the same generation from the two rotational sequences were generally similar, with the exceptions of curd firmness in F1, and RCT in F4 cows.

Table 7. Comparison between 4 generations of crossbred cows from two rotational sequences (VR-Mo-Ho and Mo-VR-Ho; dataset 2, herd B): LSM and SEM for single point milk coagulation properties and fresh cheese yield (CY) (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows, no.	Milk coagulation properties [1]					CY, %
		RCT, min	k_{20}, min	a_{30}, mm	a_{45}, mm	a_{60}, mm	
Breed combinations:							
Rotation VR-Mo-Ho:							
VR×Ho (F1)	47	19.5	4.1	40.1	44.2	42.0	17.06
Mo×(VR×Ho) (F2)	53	21.3	5.3	32.9	40.9	39.2	16.27
Ho×[Mo×(VR×Ho)] (F3)	137	20.4	4.8	34.9	42.4	40.5	16.88
VR×{Ho×[Mo×(VR×Ho)]} (F4)	60	24.8	6.0	26.6	36.1	36.2	17.51
Rotation Mo-VR-Ho:							
Mo×Ho (F1)	105	19.1	4.6	36.1	42.6	40.6	17.32
VR×(Mo×Ho) (F2)	71	21.6	5.2	29.9	40.6	38.6	17.21
Ho×[VR×(Mo×Ho)] (F3)	39	21.7	5.6	34.8	39.7	37.5	16.51
Mo×{Ho×[VR×(Mo×Ho)]} (F4)	11	18.6	4.8	33.7	37.5	35.5	16.18
SEM	-	1.2	0.4	2.7	2.1	2.1	0.37
Breed effect (*P* value)	-	0.0005	0.008	0.001	0.006	0.01	0.05
Contrast, *P* value:							
VR-Mo-Ho vs Mo-VR-Ho sequence	-	0.15	0.99	0.10	0.48	0.19	0.63
F1 vs (F2+F3+F4)	-	0.01	0.003	0.001	0.002	0.001	0.09
F2 vs (F3+F4)	-	0.93	0.96	0.62	0.23	0.24	0.93
F3 vs F4	-	0.66	0.65	0.12	0.07	0.07	0.73
Within F1	-	0.80	0.40	0.05	0.05	0.05	0.54
Within F2	-	0.83	0.96	0.33	0.88	0.74	0.03
Within F3	-	0.34	0.13	0.96	0.14	0.09	0.37
Within F4	-	0.01	0.17	0.17	0.72	0.81	0.07

[1]RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} (a_{45}, a_{60}) = curd firmness after 30 (45, 60) min from rennet addition.

DISCUSSION

In the last decade, several studies have investigated the effects of crossbreeding in dairy cattle, and have looked at different breeds and different crossbreeding sequences. The present study focused on a 3-breed rotation system in which purebred Ho heifers and cows were inseminated with semen from VR bulls, and subsequently Mo semen was used on their daughters. The latter two breeds are known for having a lower milk volume but higher milk fat and protein percentages than the Ho breed, and have been more intensively selected for the improvement of functional traits (Dezetter et al., 2015). Although other studies have investigated the effects of the same crossbreeding system on certain milk and animal traits (see, for instance, Shonka-Martin et al., 2019a, b; Hazel et al., 2017a, b; Malchiodi et al., 2014a, b), to the best of our knowledge, this is the first study to focus on the cheese-making ability of milk from crossbred cows from a long-term rotational crossbreeding system up to the fourth generation.

Comparison between Purebred Holstein and Crossbred Cows

The two herds involved in our study are representative of the two most important dairy production systems in northern Italy, where the milk is largely destined for the production of long-ripened, hard PDO cheeses, namely Grana Padano (herd A) and Parmigiano-Reggiano (herd B). The huge differences in the yield, quality and technological properties of milk from the two herds are mainly due to differences in feeding and management conditions, and also reflects the different production regulations laid down by the two PDO Consortia. Grana Padano is generally produced on large farms using many silages (especially corn silage and ear silage) and concentrates, whereas the use of silage is prohibited by the regulations governing Parmigiano-

Reggiano production, and the diets are instead based on dry forages (alfalfa and meadow hays) and some concentrates.

The slightly lower volume of milk yielded by crossbred cows compared with HO cows that we found in this study is consistent with the results of several other studies dealing with 2- and 3-breed crosses from the Ho, Mo, and VR breeds (Heins and Hansen, 2012; Malchiodi et al., 2014b; Hazel et al., 2017b; Shonka-Martin et al, 2019a). The magnitude of the difference found in the present study (-5.5% with respect to the milk yield of purebred Ho) is intermediate between the -2% reported by Hazel et al. (2017b) for Mo×Ho and VR×Ho first generation cows, and the -9% reported by Malchiodi et al. (2014b) for two generations of 3-breed crosses of Ho, Mo, and VR, and is almost identical to that reported in another multi-generation experiment using the same rotational system by Shonka-Martin et al. (2019a).

The milk produced by crossbred cows was richer in protein and casein compared with the milk from purebred Ho cows, confirming results reported by Heins and Hansen (2012), Ezra et al. (2016), and Hazel et al. (2017b), who looked at 2-breed crosses using Mo or Nordic Red sires on Ho cows, and by Malchiodi et al. (2014b) and Shonka-Martin et al (2019a), who examined 3-breed crosses of the Ho, Mo, and VR breeds.

The lower SCS of crossbred cows compared with purebred Ho observed in this study is in agreement with the findings of Heins and Hansen (2012), who compared 1st generation Mo×Ho and Scandinavian Red×Ho with purebred Ho, and of Dezetter et al. (2015), who reported lower SCS across lactation in Mo×Ho than in purebred Ho. However, Hazel et al. (2017b) reported that Mo×Ho and VR×Ho cows did not differ from Ho cows with regard to SCS during their first lactation, as did Malchiodi et al. (2014b) in a study of 3-breed crosses of the Ho, Mo, and VR breeds. Interestingly, we found the reduction in SCS associated with crossbred cows only in the most productive herd, where cows were possibly under more stressful conditions and a greater metabolic load. The response of crossbred cows and the magnitude of heterosis are known to be affected by the production environment and the level of herd production (Bryant et al., 2007; Penasa et al., 2010; Kargo et al., 2012). Concerning udder health, in a study comparing Nordic Red×Ho with purebred Ho cows Clasen et al. (2019) reported a lower incidence of mastitis in crossbred cows, particularly when they were reared in herds with a high production level, whereas there were only small or non-significant differences between crossbred and purebred cows when they were reared in herds with average or low production levels.

The average MCP traits observed in the present study were slightly worse than those reported by Malchiodi et al. (2014b) for Ho and crossbred cows; the milk samples in that study had a 3 min shorter RCT, required nearly 1 min less to reach a curd firmness of 20 mm (k_{20}), and had a 3.5 mm greater curd firmness at 30 min from rennet addition due to a better k_{CF}. The laboratory, instruments and procedures were the same in the two studies, but the herds differed, and there were also partial differences in the genetic combinations of the crossbred cows, and in the milk sample storage conditions - refrigerated in the Malchiodi et al. (2014b) study, frozen in ours.

The positive effects of crossbred cows compared with Ho cows on curd firming parameters, such as the lower k_{20} and the greater a_{30} and a_{45} are comparable with results of Malchiodi et al. (2014b), even though the sires used in that study also included Brown Swiss in addition to the Swedish Red and Montbéliarde breeds. Differences in MCP between purebred Ho and crossbred cows may be attributable to the effects of different breeds and the possible contribution of heterosis. Breed can strongly affect MCP traits (Bittante et al., 2012). Compared with Ho, the Nordic red breeds included in the Viking Red breeding program, such as the Swedish Red, have been linked with a high rate of non-coagulation and slow curd firming (Poulsen et al., 2017; Nilsson et al., 2019). Conversely, some studies have found milk from Mo to have better coagulation properties than milk from Ho cows (Macheboeuf et al., 1993; Bittante et al., 2012). Among-breed differences in MCP may be partially explained by differences in genetic variants of milk protein content (Bittante et al., 2012). In this regard, milk from crossbred cows sired by Swedish Red and Mo bulls has been found to have higher κ-casein contents and proportions than milk from Ho cows (Maurmayr et al., 2018), which is consistent with the favorable curd firmness properties of the milk from crossbreds in the present study. Finally, it is well known that heterosis can be important and positive for yield traits, but may have negligible effects on nutrient contents (Dezetter et al., 2015). There is currently no literature on the effects of heterosis for MCP traits, and the data from the present study are not suitable for estimating heterosis because we did not include pure VR and Mo cows for comparison.

Interestingly, the favorable effects of crossbred cows compared with Ho cows on curd firming parameters were particularly evident in the herd with the greatest milk yield, but also the worst MCP traits. In large surveys of commercial dairy farms, dairy system was found to affect MCP (Bittante et al., 2015); moreover, an increase in herd productivity has been related to delayed coagulation time but not reduced curd firmness (Stocco et al., 2017). Clasen et al. (2019) reported that the response of F1 Nordic Red×Ho cows compared with purebred Ho cows was independent of the production level of the herds, but they were examining yield and some functional traits, not the coagulation properties of the milk.

The efficiency of the cheese-making process depends on CY as well as the recovery of milk nutrient constituents in the curd and their loss in the whey (Banks, 2007). Given the increasing use of milk for cheese production in many countries, factors affecting the CY of milk are of relevance to the profitability of the dairy sector. In the present study, we found the average CY of curd to be generally greater than that reported in other studies using laboratory procedures for individual cheese manufacturing (Cipolat-Gotet et al., 2016; Stocco et al., 2018), mainly due to greater water retention in the curd. Regarding the proportion of milk nutrients retained in the curd, the average $REC_{PROTEIN}$ obtained in this study is fully consistent with that reported by the above-mentioned authors, whereas the average REC_{FAT} is much lower. Variations in these parameters have been associated with both animal-related factors, such as breed, parity, stage of lactation, feeding and management, and cheese-making conditions, such as milk storage before processing and the cheese-making procedures (Cipolat-Gotet et al., 2016). With regard to storage, the milk samples in the present study were frozen before being processed into cheese, whereas in other studies the milk was refrigerated before processing. By freezing the samples, we were able to sample a larger number of cows and to remove the effect of the day/season of sampling, even though it resulted in a greater loss of fat in the whey. These conditions may therefore affect the comparison between our absolute CY and REC_{FAT} values and those of other trials, but not the comparison among different breed combinations within this study, as the milk-storage conditions and processing were the same for all the samples.

Breed has been shown to greatly affect CY traits, and differences among several dairy and dual-purpose breeds have been reported (Stocco et al., 2018). In previous large surveys (Cecchinato et al., 2015; Stocco et al., 2018), we found that the technological aptitude of milk from the Italian Simmental, a breed with many genetic exchanges with the French Montbéliarde, was better than that of Ho cows. Similarly, Martin et al. (2009) reported that milk from Mo had a greater CY than milk from Ho cows, although others found no differences between the two breeds (Verdier-Metz et al., 1998). Unfortunately, there is no information regarding comparisons of different breed combinations nor regarding the role of heterosis on CY traits.

The farms differed for CY_{CURD} mainly due to differences in the amount of water retained in the curd. Stocco et al. (2018) reported that herd was an important source of variation in CY_{CURD}, and that differences among herds were larger for water than for solid retention in curd. The interaction between the effects of herd and of crossbreeding was significant for CY_{CURD}, but it was only a tendency for REC traits (Table 5). Again, the crossbred cows were superior to the Ho cows in the herd with the highest production level, but not in the other.

Comparison among Crossbred Cows

For the long-term management of crossbreeding as a systematic mating procedure on dairy farms, a three-breed rotational crossbreeding program has been recommended as an optimal strategy for dairy herds (Sørensen et al., 2008). Indeed, compared with the use of only two breeds, using three breeds in rotational crossing systems results in a greater level of heterosis (Hazel et al., 2014; Sørensen et al., 2008). However, most studies in the literature dealing with crossbreeding in dairy cows have compared the performances of purebred (usually Ho) and first generation crossbred cows, but few have assessed the performance of the second or subsequent generations within a rotational crossbreeding system and compared the outcomes of crossbred cows of different crossbreeding generations. Recently, Shonka-Martin et al. (2019a) compared cows obtained from a 3-breed rotational crossbreeding system involving Mo, VR and Ho breeds and purebred Ho, but the study was concerned with comparing the purebreds and crossbreds, and the results regarding cows from the different crossbred generations were not reported. In a comparison of purebred, F1 crossbred and backcross cows from Ho, Mo and Normande breeds, Dezetter et al. (2015) found recombination estimates that were consistently negative, but characterized by large standard errors, so they never differed significantly from zero.

In this study, crossbred cows of different generations were similar in terms of the yield, composition and cheese-making properties of milk, with only some sporadic differences, despite large differences in their breed composition, as the genes from the Ho breed accounted for 50%, 25%, 62.5% and 31.25% of the entire genome of cows of the F1, F2, F3, and F4 generations, respectively. Heterosis was correspondingly 100%, 100%, 75% and 87.5% of the maximum level. The only exception regarded the overall better MCP of F1 cows compared with crossbreds of the following generations (F2+F3+F4). Comparisons of crosses from the two rotational sequences within each generation also showed them to perform very similarly, with, again, the slightly better curd firmness traits in the milk from VR × Ho than in that from Mo × Ho F1 cows as the main exception. Malchiodi et al. (2014b) also found no differences in milk yield and composition, and SCS between F1 and F2 crossbred cows from Swedish Red, Mo, and Brown Swiss sires, and between different crosses within each generation. Moreover, Malchiodi et al. (2014b) reported some differences in MCP traits between different crosses within both the first and the second crossbred generations, but not between the first and the second generations. However, that study only looked at two generations of crossbred cows, and also included Brown Swiss as a sire breed, but not backcrosses to Ho (F3) in the genetic lines examined.

Lastly, the slight, sporadic differences found between the two rotational crossbreeding sequences within each crossbred generation explain why the sire-breed sequence used in the 3-breed rotational program did not affect its overall outcome. Therefore, crossbred cows produced more or less the same volume of milk and cheese regardless of whether the mating program started with the VR or Mo sire breed, or of the generation reared, and this may be a useful practical indication for farmers adopting this crossbreeding program.

CONCLUSIONS

Crossbreeding is of interest to commercial dairy farmers to improve the robustness of their dairy cows, thereby enhancing their health status, fertility and longevity. A three-breed rotational crossbreeding program has been proposed as an effective strategy for systematic crossbreeding in dairy farms. This study adds new knowledge about the long-term effects of such a program on traits that have received less attention in the literature, such as the technological properties and cheese-making ability of milk, and has taken into account the performance of crossbred cows up to the fourth generation of the rotational crossbreeding system. Data from this study confirm that crossbred cows yield a lower volume of milk compared with purebred Ho, but the milk protein and casein contents were higher and the SCS lower. Milk from crossbred cows also had slightly better milk coagulation and curd firming properties than the milk of purebred Ho, but there were no significant differences in CY and milk nutrient recovery in the curd. The 3-breed rotational breeding system using Mo, VR and Ho sires can therefore also be implemented in farming systems specializing in cheese production. The favorable response of crossbred cows compared with purebred Ho cows with respect to SCS, curd firming properties, and also CY was greater and more evident in the herd with the highest production level and using many silages in the diet, where cows may have a greater metabolic load. However, speculation that there may be interactions with production systems in the response of crossbred cows needs further investigation. Crossbred cows of different generations and breed rotation sequences performed similarly, with the only exception of the better curd firming properties of milk from F1 crossbred cows; reciprocal crosses within each crossbred generation were also fairly similar in milk quality, and technological and cheese yield traits. Therefore, whether the VR or the Mo sire breed was used to produce F1 cows at the start of the rotational crossbreeding program, had no practical implications for the overall performance of the program, and there are no appreciable changes in the yield, composition and cheese-making ability of milk, and the proportions of milk nutrients retained in the cheese in the subsequent crossbreeding generations.

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APPENDIX

Supplemental Table S1. Descriptive statistics of milk yield and composition, single point milk coagulation properties and curd firming (CF_t) equation parameters, cheese yield and milk nutrient recovery in curd (REC) for dataset 1 (all purebred Ho and three generations of 3-breed rotational [VR-Mo-Ho] crossbred cows from herd A and herd B) and for dataset 2 (four generations of two 3-breed rotational [VR-Mo-Ho and Mo-VR-Ho] crossbred cows of herd B only)

Trait	Dataset 1			Dataset 2		
	Cows, no.	Mean	SD	Cows, no.	Mean	SD
Milk yield, kg/d	829	33.6	10.22	523	30.0	9.74
Milk composition:						
Fat, %	816	3.81	0.87	508	3.80	0.81
Protein, %	816	3.67	0.39	508	3.75	0.34
Casein, %	825	2.94	0.33	518	3.04	0.29
Lactose, %	824	5.07	0.20	518	5.03	0.19
SCS[1]	825	2.75	1.74	513	2.60	1.57
Milk coagulation properties[2]:						
RCT, min	795	23.9	10.1	511	21.1	8.0
k_{20}, min	729	6.1	3.5	500	5.1	2.8
a_{30}, mm	641	30.9	16.4	466	33.6	16.5
a_{45}, mm	749	36.0	13.7	504	41.0	12.6
a_{60}, mm	794	33.7	12.6	511	39.4	12.0
CF_t[3] equation parameters:						
RCT_{eq}, min	795	24.3	9.9	511	21.6	7.9
k_{CF}, % × min^{-1}	794	9.2	2.9	513	9.2	2.9
k_{SR}, % × min^{-1}	796	1.1	0.6	513	1.2	0.5
CF_p, mm	794	57.7	20.3	511	66.7	17.1
CF_{max}, mm	794	38.6	13.6	512	44.5	11.5
t_{max}, min	796	47.1	10.1	513	46.4	9.8
Cheese yield (%):						
Curd	800	16.98	2.41	518	17.09	2.37
Solids	793	4.81	0.81	510	4.89	0.76
Water	797	12.15	1.99	512	12.18	1.96
REC (%):						
Protein	791	80.06	2.52	510	80.23	2.52
Fat	776	56.76	13.21	494	54.36	13.16

[1]SCS = 3 + log2 (SCC/100,000).
[2]RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} (a_{45}, a_{60}) = curd firmness after 30 (45, 60) min from rennet addition.
[3]RCT_{eq} = RCT estimated according to curd firm change over time modeling (CF_t); k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_P = asymptotic potential curd firmness; CF_{max} = maximum curd firmness; t_{max} = time to reach CF_{max}.

Supplemental Table S2. Comparison between 4 generations of crossbred cows from two rotational sequences (VR-Mo-Ho and Mo-VR-Ho; dataset 2, herd B): LSM and SEM for curd firming (CF_t) equation parameters (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cows, no	Curd firming traits[1]					
		RCT_{eq}, min	k_{CF}, % × min^{-1}	k_{SR}, % × min^{-1}	CF_p, mm	CF_{max}, mm	t_{max}, min
Breed combinations:							
Rotation VR-Mo-Ho:							
VR×Ho (F1)	47	20.1	10.1	1.2	71.5	48.7	44.1
Mo×(VR×Ho) (F2)	53	21.5	9.0	1.1	63.4	42.9	46.8
Ho×[Mo×(VR×Ho)] (F3)	136	20.9	9.5	1.1	67.5	45.9	46.2
VR×{Ho×[Mo×(VR×Ho)]} (F4)	60	25.3	9.5	1.1	59.7	40.6	48.5
Rotation Mo-VR-Ho:							
Mo×Ho (F1)	105	19.5	9.8	1.2	68.1	46.2	44.9
VR×(Mo×Ho) (F2)	71	22.7	9.4	1.1	63.9	42.8	46.9
Ho×[VR×(Mo×Ho)] (F3)	37	23.1	9.5	1.1	62.5	42.6	47.5
Mo×{Ho×[VR×(Mo×Ho)]} (F4)	11	19.2	11.2	1.4	62.8	42.8	40.4
SEM		1.3	0.6	0.1	2.9	1.9	1.6
Breed effect (*P* value)		0.0003	0.26	0.07	0.0005	0.0003	0.05
Contrast, *P* value:							
VR-Mo-Ho vs Mo-VR-Ho sequence		0.36	0.11	0.10	0.48	0.43	0.15
F1 vs (F2+F3+F4)		0.01	0.38	0.11	0.0001	<0.0001	0.14
F2 vs (F3+F4)		0.97	0.06	0.13	0.80	0.96	0.33
F3 vs F4		0.86	0.09	0.08	0.18	0.19	0.16
Within F1		0.99	0.19	0.10	0.08	0.08	0.28
Within F2		0.42	0.40	0.41	0.85	0.95	0.94
Within F3		0.12	0.90	0.45	0.07	0.08	0.45
Within F4		0.02	0.05	0.03	0.52	0.50	0.006

[1]RCT_{eq} = Rennet coagulation time estimated according to curd firm change over time modeling; k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness; t_{max} = time to reach CF_{max}.

CHAPTER 4

Milk coagulation traits, cheese yield, rheological, chemical and sensory attributes of model cheeses from purebred Holstein and three-breed rotational crossbred cows from Viking Red, Montbéliarde, and Holstein sires

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ABSTRACT

Crossbreeding in dairy cattle is becoming popular as a possible strategy to counter the decline of fertility, health and longevity of purebred cows, namely Holstein. However, studies dealing with the effects of crossbreeding on cheese production and quality of products are sparse. In the present study, 3-breed rotational crossbred cows from Viking Red (**VR**), Montbéliarde (**Mo**) and Holstein (**Ho**) sires over four generations and purebred Ho were compared for milk composition, casein micelle size, fat globule size (d_{43}, d_{32}), coagulation properties (**MCP**), cheese yield (**CY**), rheological, chemical and sensory properties of cheese. Individual milk samples (2000 mL each) were collected from 120 multiparous cows (Ho: 40; CR: 80, 20 for each generation from F1 to F4) during the evening milking of 6 following weekly sampling sessions (20 cows sampled/each milking session). Milk samples were analyzed for milk composition, casein micelle size, fat globule size, MCP, CY (using 1,500 mL of raw full-fat milk), milk nutrient recovery in the curd (**REC**). The prepared cheeses were evaluated for weight, ripening loss, rheological, chemical and sensory attributes. The sensory properties of the ripened cheese were assessed by a panel of 6 trained panelists. Compared with that of Ho cows, milk from CR cows had greater content of protein and casein and lower lactose and SCS. Moreover, milk from CR cows had smaller average casein micelle size compared to milk from Ho cows, with comparable, although tendentially smaller, fat globule size (d_{43}, d_{32}). Also, milk from crossbred cows showed slightly more favorable curd firming rate (k_{20}) and greater curd firmness at 45 and 60 min after rennet addition. Milk from Ho and CR cows evidenced very similar CY and REC traits. Moreover, weight, chemical composition, rheological (color, texture) and sensory attributes of produced model cheese were comparable for Ho and CR cows. Among CR cows, VR sired crossbred cows exhibited a positive effect on milk composition (protein and casein), curd firming properties, CY, REC traits and weight of fresh curd and 70 d-ripened cheese, whereas Mo sired crossbred cows had opposite trends for these traits. In conclusion, our findings indicates that rotational crossbreeding scheme did not exert any negative effect on cheese production and quality and seems to be suitable also in the farming system focused in typical cheese production.

Key words: dairy cow, crossbreeding, cheese production, cheese quality

INTRODUCTION

Crossbreeding in commercial dairy production is raising a growing interest due to declining fertility, health and longevity of modern dairy cows (Hazel et al., 2017b). Another reason is to counteract the increasing rate of inbreeding, which is a problem of some dairy breeds, mainly the Holstein Friesian (Sørensen et al., 2005; Hazel et al., 2017b). Thus, the use of crossbreeding in dairy cattle is increasing globally (Hazel et al., 2017b; Clasen et al., 2019). For the long-term management of crossbreeding as a systematic mating procedure on dairy farms, a three-breed rotational crossbreeding program has been recommended as an optimal strategy for dairy herds (Sørensen et al., 2008). Indeed, compared with the use of only two breeds, using three breeds in rotational crossing systems results in a greater level of heterosis (Sørensen et al., 2008; Hazel et al., 2014).

A three-breed rotational crossbreeding program has been started in commercial dairy farms in the last decades, which is commercially named as **ProCROSS**, managed by Coopex Montbèliarde (Roulans, France) and Viking Genetics (Randers, Denmark). This program, involving three breeds, the Holstein (**Ho**), the Montbéliarde (**Mo**) and the Viking Red (**VR**) have risen interest particularly in dairying systems with limited opportunity of grazing (Dechow and Hansen, 2017).

Numerous studies have reported the benefit of crossbreeding for production, milk quality, (Heins and Hansen, 2012; Malchiodi et al., 2014b; Ezra et al., 2016; Hazel et al., 2017b), health, fertility and longevity of dairy cattle (Heins et al., 2012; Malchiodi et al., 2014a; Hazel et al., 2017a) and body traits (Blöttner et al., 2011; Saha et al., 2018). Nonetheless, the information on cheese making properties of crossbreed cows is scarce. Particularly, no previous studies have been focused on the variability of rheological, chemical and sensory traits of cheese produced from milk of crossbred cows.

Cheese is considered one of the major dairy products in worldwide, and Europe, with nearly 50% of the world production, is the world top producer (US Department of Agriculture; Economic Research Service, 2018). Therefore, all the features of cheese making process from herd to consumer level such as milk quality, milk coagulation properties, cheese yield, rheological, chemical and sensory traits of cheese are important for dairy industry. Especially, these are also vital for the Protected Designation of Origin (**PDO**) cheese production chain where systematic control and regulation are followed in their supply chain (Bittante et al., 2011). In our previous research, we have investigated the milk coagulation traits and cheese yield ability of crossbred cows using frozen milk samples and a laboratory coagulation method based on 9 mL of milk from 1116 animals. In the present study, we have used an individual model cheese making process from refrigerated milk with planned design of purebred Ho and crossbred cows for investigating the rheological, chemical and sensory traits of cheese. Focusing on the milk composition, cheese making properties coagulation, cheese yield, and rheological, chemical and sensory traits of cheese, the objective of this study was to compare the purebred Ho to 3 breed rotational crossbred cows from VR, Mo and Ho.

MATERIALS AND METHODS

Herd, Animals and Crossbreeding Scheme

This study involved 120 lactating cows (40 Holstein and 80 crossbreds) reared in a dairy herd located in northern Italy and following a three-breed rotational crossbreeding scheme (ProCROSS) for several years. The basic scheme was based on the use of VR semen on Ho cows (F1: VR×Ho), n = 20; the use of Mo semen on F1 crossbred cows to produce F2 Mo×(VR×Ho), n = 20; and the use of Ho semen on F2 cows to develop third generation crossbred cows F3 (Ho×[Mo×(VR×Ho)], n = 20). Furthermore, F3 cows were inseminated using VR semen to obtain F4 VR×{Ho×[Mo×(VR×Ho)}] cows, n = 20. This herd followed a management system according to the rules of PDO Parmigiano Reggiano cheese, where silage were not allowed for feeding and the ration (fed as TMR) was based on dry roughage, mainly alfalfa and meadow hay, and concentrate.

Milk Sampling and Milk Yield and Composition

Individual milk samples (2,000 mL) were collected from 120 purebred Ho and crossbred cows during the evening milking of six weekly sampling sessions hold from January to February. Each session involved 20 purebred Ho and four generations crossbred cows. The sampled cows yielded 32.1 ± 7.9 kg/d milk. The average days in milk were 165 ± 49 d and the parities number was 2.83 ± 0.9 for sampled cows. After collection, milk samples (without preservative) were refrigerated (4^{o}C) and transferred to the milk quality laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) at the University of Padova, Italy for analyses. All samples were analyzed and processed within 24 h of collection.

Milk yield data were obtained from the Italian official recording system. Milk compositions (fat, protein, casein and lactose) were measured with a Milkoscan FT2 infrared analyser (Foss Electric A/S, Hillerød, Denmark). The milk pH was measured after the heating step of the cheese making process, using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Somatic cell count (SCC) values were obtained from the Fossomatic Minor FC counter (Foss Electric A/S, Hillerød, Denmark) and then converted to somatic cell score (**SCS**) by log transformation (Ali and Shook, 1980). The distribution of casein micelle size was measured by laser light scattering (Mastersizer 2000 with a Hydro 2000 SM sampling unit, Malvern Instruments, Malvern, UK) according to the detail procedure described by Poulsen et al. (2017). The size of milk fat globule was also determined by laser scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) according to Ménard et al. (2010). In brief, the refractive index was considered 1.46 and absorbance coefficient wave length was fixed 0.0001 for milk fat globule size. Then, 100 μL of milk were poured into the water contained measurement cell of apparatus and maintaining the obscuration rate between 10 to 12%. After that, 1 mL of 35mM EDTA/NaOH pH 7.0 buffer solutions was added to the measurement cell to dissociate casein micelles for ensuring that the measured particle size distribution was fat globule. All analyses were performed in triplicate. Fat globule size: average diameter (d_{43}) and surface diameter (d_{32}) was calculated automatically by installed software in the apparatus.

Milk Coagulation and Curd Firmness Properties

Milk coagulation properties (**MCP**) were assessed in duplicate for each cow by mechanical lactodynamograph (2 instruments; Formagraph, Foss Electric A/S). Milk samples of 10 mL were heated at 35°C and then mixed with 200 μL of rennet solution (Hansen Standard 215 with 80 ± 5 % chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2 % (wt/vol) in distilled water. The observations of milk coagulation traits in the lactodynamographs lasted 60 min and provided the following traditional MCP traits: rennet coagulation time (RCT, min); time to reach curd firmness 20 mm (k_{20}, min) and curd firmness after 30, 45 and 60 min (a_{30}, a_{45} and a_{60}, mm). As curd firmness (**CF**) was assessed every 15 s for 60 min, 240 CF individual point observations were recorded for each replicate. Afterwards, all the curd firmness observations from each milk samples were modeled to estimate the individual curd firming and syneresis equation parameters proposed by Bittante et al. (2013), and namely the curd firmness at time t (CF_t, mm); the asymptotical potential value of CF at an infinite time (CF_p mm); the curd firming instant rate constant (k_{CF}, % / min); the syneresis instant rate constant (k_{SR}, %/min) and the estimated RCT (RCT_{eq}, min).

Individual Model Cheese Making Procedure and Cheese Yield Traits

For performing the individual model cheese-making procedure 2 water baths facilities with a digital temperature controller and pumps for mixing the water for homogenous heat distribution throughout the water baths were used. Each water bath had the place for five stainless steel vats (capacity 1,500 mL). Twenty (2 water baths × 5 vats × 2 times) individual milk samples were processed in one session per week. Detailed procedures of micro-cheese making procedure can be found in Stocco et al. (2018).

Briefly, each raw full-fat milk sample (1,500 mL) was poured into a stainless steel vat and heated at 35°C in water bath for 30 min, and then pH was recorded. Commercial rennet [Hansen Standard 215, with 80 ± 5 % chymosin and 20 ± 5 % pepsin; 215 international clotting units (IMCU)/mL (Pacovis Amrein AG, Bern, Switzerland)] was diluted to 4.29% in distilled water and 8 mL of rennet solution was added to each stainless steel vat. After coagulation, the curd was cut and whey separated from the curds is drained off, then whey was measured for chemical analysis using a MilkoScan FT2 (Foss). The curd was pressed with a cheese pressing machine for 30 min at 250 kPa with turning every 10 min and then soaked in a brine solution (20% NaCl) for 30 min. After brining, the cheese wheels were weighed and pH was recorded using a Crison Basic 20 electrode (Crison Instruments SA, Barcelona, Spain). Using the weight and composition of the milk and the whey, three CY traits (CY_{CURD}, CY_{SOLIDS} and CY_{WATER}) and four milk nutrient recoveries in curd traits ($REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS} and REC_{ENERGY}) were determined in percentage according to Cipolat-Gotet et al. (2013).

The prepared cheese wheels were transferred in the oven for ripening at 15^{o}C and 85% relative humidity for 7 days. After that, cheese wheels were weighed and kept in vacuum seal plastic bags and left to ripen at 15^{o}C and 85% relative humidity for 63 days. At the end of the ripening, cheeses were opened from vacuum seal plastic bags and weighed. Cheese yield after 70 days was calculated as the ratio of cheese weight and the weight of the processed milk.

Cheese Weight, Rheological and Chemical Composition of Cheese

The 70-d ripened cheeses were analyzed for rheological traits and chemical composition. At the end of 70 d ripening, the vacuum seal plastic bags were opened and each cheese wheel was weighed. At first, rind of cheese was removed and later each cheese wheel was cut into two equal parts: one part was used for rheological traits and chemical composition and the other part was used for sensory analysis. For chemical composition, each aged cheese sample was analyzed for DM, fat, protein (N × 6.38) and ash contents according to IDF (International Dairy Federation) standards [4A:1982 (IDF, 1982), 5B:1986 (IDF, 1986), 25:1964 (IDF, 1964a) and

27:1964 (IDF, 1964b). The pH (acidity) of the cheese was measured by using a Crison Basic 25 pH meter with a 5053T electrode (Crison Instruments SA, Barcelona, Spain).

Rheological properties of cheese include the instrumental color and texture analysis. The cheese color was determined with a Minolta colorimeter (CM-500d, D65 illuminant and 10^{o} observer; Konica-Minolta Sensing Inc., Ramsey, NJ) in 3 consecutive readings (the average was considered) and expressed in terms of L* (Lightness, ranges from 0 = black to 100 = white), a* (positive values indicates red and negative values indicates green) and b* (positive values indicates yellow and negative values indicates blue).

Texture properties of individual model cheese were determined by the texture profile analysis process which was conducted using a TA.XTplus texture analyzer (Stable Micro Systems, London, England). All analyses were performed in controlled room temperature (20^{o}C). A plate (diameter 35 mm) compressed a cylinder shape cheese (1 cm^{2} area and 20 mm height), placed on a fixed table. The considerable force load was 500 N in a load cell. Then each cheese sample was compressed (25% compression) using a crosshead speed 2 mms^{-1}. After completed the compression stroke, plunger abruptly worked in reversed direction and started an upward stroke at 2 mms^{-1}. After waiting for 5 s and a second compression (up and down cycle) run on the same sample. All operations were automatically controlled by the texture analyzer and data were also recorded automatically in the instrument. The parameters of hardness, cohesiveness, elasticity and chewiness were calculated by the software instrument called Texture Exponent (Stable Micro System, London, England). Hardness and chewiness were displayed in Newton (N) and elasticity in millimeter (mm) scale.

Sensory Attributes of Cheese

A group of 6 panelists, all females, and 30 ± 2 years old was used to evaluate the sensory attributes of cheese. The panelists were previously trained for two trial sessions to improve their skills on 2 months ripened Caciotta cheese, which is a cheese with characteristics comparable to our ripened cheeses in terms of color, texture, taste, smell, and flavor. Besides, other dairy products were used to train the panel: yogurt for milky and lactic acid flavor, milky and both fresh and seasoned cheeses to compare attributes with higher and lower intensity. As in each day the panel evaluated 10 different cheeses, two sensory evaluation sessions were considered in a week. Total cheese evaluation period was 6 weeks (12 session total, 2 sessions per week, and 90 minutes per session) from April to mid-May, 2019. Testing sessions were performed in the morning, under normal light conditions, at room temperature (22 ± 1^{o}C), and in the day interval between 11.30 and 13.30. Cheese samples (2 pieces, size: about 1 cm thick × 3–4 cm wide × 5–6 cm long) without rind were coded with sample number with three casual digits kept on petri dishes and served on random order. In addition, water and unsalted crackers were supplied to wash the mouth between each cheese sample evaluation.

Briefly, panelist made their sensory evaluation of cheese in accordance with Bérodier et al. (1997) and Lavanchy et al. (1999) on the basis of following sensory descriptors classification: color, texture descriptors (elasticity, firmness and moisture) smell intensity, flavor intensity, taste descriptors (sweet, salt, sour, bitter and umami). Each attribute was scored according to a scale ranging between 1 (low) to 10 (high) anchored with standard of food references for cheese (Lavanchy et al., 1999).

Statistical Analysis

Data from 120 cows were used to prepare two data sets: 1) milk and cheese related traits, 2) sensory traits of cheese. Before analysis, all data were classified for parity (**PAR**, 2 classes: 2nd parity and > 2nd parity), days in milk (**DIM**, 3 classes: class 1: < 150d; class 2: 151 to 210 and class 3: > 210 d) and breed combinations (5 classes: purebred Ho, and F1, F2, F3, and F4 crossbred cows). Milk and cheese related traits (milk composition, casein micelle size, fat globule size, milk coagulation and curd firming equation parameters, cheese yield and milk nutrient recovery traits, cheese weight, composition and rheological traits) were analyzed using a mixed model procedure of SAS 9.4 (SAS Institute, 2013), considering the random effects of sampling date and the fixed effects of PAR, DIM and breed combinations.

Data from sensory traits of cheese were analyzed by a mixed model (SAS Institute, 2013), which included the effect of random effects of sampling data, panelists and animal within the breed, and the fixed effects of order of cheese presentation, PAR, DIM and breed combinations.

Orthogonal contrasts ($P < 0.05$) were used in both datasets to observe the following effects: the effect of crossbreeding, that is purebred Ho vs crossbred cows; the effects of VR sire, that is (F1+F4) *vs* (F2+F3); the effects of Mo sire, that is F2 *vs* (F1+F3+F4); and the effects of Ho sire, that is F3 *vs* (F1+F2+F4).

RESULTS

Descriptive Statistics of Milk, Cheese and Sensory Traits

Descriptive statistics of composition, quality, cheese-making properties of milk and composition and rheological traits of cheese are presented in Table 1. Average milk fat, protein, casein and lactose contents were 4.34, 3.95, 3.15 and 5.00%, respectively. The observed coefficient of variation (CV) for milk fat (27.45%) was greater than observed for other milk composition traits, whereas SCS had the highest CV (57.69%). The mean values of pH, SCS and casein micelle size were 6.62, 2.79 and 124.7 nm, respectively. The volume weighted mean (d_{43}) and surface (d_{32}) diameters of measured fat globule were 3.81 and 3.33 μm, respectively. Regarding milk coagulation traits (MCP and curd firming equation parameters), CV varied from 18.10 (CF_p/CF_{max}) to 45.87 % (k_{20}). The fresh curd yield was nearly 177 g/kg of milk with 40% of solids and 60% of water. The average fresh curd weight was 268 g and cheeses weighed on average 157 g after 70 d of ripening. The ripened cheese contains 46.0% fat and 39.8% protein on DM basis. The observed CV for cheese composition traits was around 10%. Regarding the sensory profile (Table 2), the prepared cheeses were whitish appearance (2.82) with strong flavor (6.05) and smell (5.60), and sour (6.16) dominant in taste with respect to sweet (2.69).

Table 1. Descriptive statistics of milk composition, single point milk coagulation properties (MCP), curd firming (CF_t) equation parameters, yield of fresh curd and milk nutrient recovery in fresh curd (REC), weight, ripening loss and composition and rheological traits of ripened cheese (n=120)

Trait	Mean	SD	CV	Percentile	
				1st	99th
Milk composition:					
Fat, %	4.34	1.19	27.45	2.23	7.63
Protein, %	3.95	0.36	9.08	3.22	5.02
Casein, %	3.15	0.26	8.27	2.61	3.77
Lactose, %	5.00	0.18	3.60	4.63	5.44
pH	6.62	0.06	0.89	6.51	6.76
SCS[1]	2.79	1.61	57.69	-0.18	6.46
Casein micelles size, nm	124.7	4.01	3.22	118.5	138.8
Milk fat globule size[2]:					
d_{43}, µm	3.81	0.44	11.47	2.78	4.87
d_{32}, µm	3.33	0.37	11.16	2.51	4.29
Single point MCP[3]:					
RCT, min	24.4	7.1	29.2	12.7	39.8
k_{20}, min	5.9	2.7	45.9	2.5	12.7
a_{30}, mm	28.2	15.1	53.3	0.5	55.7
a_{45}, mm	36.3	10.8	29.8	10.5	56.2
a60, mm	38.0	7.3	19.1	22.0	54.7
CF_t equation parameters[4]:					
RCT_{eq}, min	25.0	6.9	27.8	13.1	40.3
k_{CF}, % × min^{-1}	9.1	3.0	32.4	5.5	17.4
k_{SR}, % × min^{-1}	0.8	0.4	55.5	0.0	1.8
CF_p, mm	57.6	10.4	18.1	34.5	78.7
CF_{max}, mm	42.0	7.6	18.1	25.2	57.4
t_{max}, min	49.3	9.7	19.7	28.6	60.0
Yield of (%):					
Fresh curd (FC)	17.68	2.59	14.63	12.48	24.20
Solids in FC	7.07	1.18	16.64	4.94	10.33
Water in FC	10.60	1.69	15.96	7.01	14.82
$Cheese_{70D}$[5]	10.37	1.53	14.78	7.06	14.14
REC in FC (%):					
Protein	77.59	1.95	2.52	72.11	81.60
Fat	79.96	6.23	7.79	59.88	89.26
Solids	50.15	4.40	8.77	41.32	60.93
Energy	63.84	4.66	7.31	52.86	72.53
Cheese weights and loss:					
Fresh curd weight, g	268	39.1	14.6	188	364
$Cheese_{70D}$ weight, g	157	23.2	14.7	107	213
Ripening loss, %	41.3	2.82	6.84	34.3	49.1
Cheese composition:					
DM, %	68.7	2.54	3.70	64.0	74.3
Protein, % of DM	39.8	3.97	10.0	31.5	49.8
Fat, % of DM	46.0	4.98	10.8	31.8	55.9
Ash, % of DM	5.98	0.75	12.6	4.76	7.86
pH	5.09	0.21	4.03	4.59	5.55
Cheese rheological traits[6]:					
L*	81.5	3.18	3.90	73.7	87.4
a*	-0.84	0.62	-74.7	-2.01	0.71
b*	14.4	1.54	10.7	11.0	17.6
Hardness, N	25.38	9.24	36.4	7.57	47.51
Cohesiveness	0.72	0.06	8.26	0.58	0.83
Elasticity index	0.83	0.07	7.85	0.64	0.92
Chewiness, N	15.23	6.64	43.6	1.76	35.68

[1]SCS = $\log_2$(SCC/100,000) + 3

[2]Milk fat globule size: d_{43} = Average diameter; d_{32} = Sauter diameter

[3]Single-point MCP: RCT = rennet coagulation time; k_{20} = curd firming rate as min to a curd firmness of 20 mm; a_{30} (a_{45}) = curd firmness after 30 (45) min from rennet addition.

[4]CFt equation parameters: RCT_{eq} = rennet coagulation time estimated from the equation; CF_P = asymptotic potential curd firmness; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness attained within 45 min; t_{max} = time to reach CF_{max}.

[5]CY_{70D} = cheese yield after 70 d of ripening

[6]L* = Lightness, a* = redness/greenness, b* = yellowness/bluness

Table 2. Descriptive statistics of cheese sensory scores (score from 1 to 10, from low to high, n=720)

Sensory Traits	Mean	SD	CV
Color	2.82	1.26	44.62
Elasticity	2.62	1.62	61.82
Firmness	3.98	1.95	48.92
Moisture	2.07	1.12	54.16
Smell	5.60	1.93	34.49
Flavor	6.05	1.79	29.59
Sweet	2.69	1.75	64.97
Salt	4.74	2.19	46.27
Sour	6.16	1.98	32.10
Bitter	4.02	2.89	71.82
Umami	5.15	1.66	32.26

Milk Composition, Single Point MCP and Curd Firming Equation Parameters

Compared with Holstein milk, milk from crossbred cows had comparable fat content, but greater protein and casein contents, and lower ($P < 0.05$) in lactose content and SCS (Table 3). Among different sired crossbred cows, VR sired crossbred cows showed greater protein and casein content in milk with lower lactose content and SCS. Crossbred cows originated from Mo sire, on the contrary, had lesser protein and casein content in milk, whereas Ho sired crossbred cows tended to have lower protein but greater lactose content in milk than other sired crossbred cows. Casein micelle size from the milk of crossbred cow's was smaller than Holstein's milk (124 nm *vs* 126 nm). Among the crossbred cows, milk from Mo sired cows tended to provide milk with a lower casein micelle size. Milk fat globule size is often reported as volume weighted mean (d_{43}) and surface (d_{32}) diameters, and samples are described also in terms of share (small, medium and large) in accordance of fat globules ranging in size < 3 μm, 3-6 μm and > 6 μm, respectively. Although, the fat globule size for d_{43} and d_{32} did not differ between purebred Ho and crossbred cows, but tendential smaller fat globule size (d_{43}, d_{32}) reported in crossbred cows (Table 3). Different sired crossbred cows had comparable milk fat globule size. The share of small fat globules tended to slight higher for crossbred cows (29.9%) than Ho (27.3%) reported in Figure 1.

Table 3. Least squares means and standard error of the means (SEM) for milk composition, pH, somatic cell score (SCS), casein micelle size and fat globule size across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Milk composition traits, %							Fat globule size[2]	
	Fat	Protein	Casein	Lactose	pH	SCS[1]	Casein micelle size, nm	d_{43}, µm	d_{32}, µm
Breed:									
Purebred Holstein (Ho)	4.40	3.90	3.11	5.03	6.61	3.30	125.8	3.87	3.36
VR×Ho (F1)	4.56	4.16	3.32	4.95	6.61	2.30	123.8	3.77	3.34
Mo×(VR×Ho) (F2)	4.21	3.90	3.10	4.94	6.64	2.91	123.0	3.77	3.28
Ho×[Mox(VR×Ho)] (F3)	4.23	3.92	3.15	5.03	6.61	3.07	125.3	3.65	3.23
VR×{Ho×[Mo×(VR×Ho)]} (F4)	4.55	4.15	3.26	4.90	6.62	2.42	124.7	3.76	3.29
SEM	0.28	0.08	0.06	0.04	0.02	0.39	1.03	0.10	0.09
Breed effect (*P v*alue)	0.84	0.01	0.008	0.007	0.45	0.10	0.05	0.47	0.77
Contrasts, *P* value:									
Ho vs crossbreds (F1+F2+F3+F4)	0.94	0.03	0.03	0.008	0.75	0.03	0.03	0.12	0.32
VR sire: (F1+F4) vs (F2+F3)	0.24	0.002	0.004	0.09	0.54	0.08	0.92	0.61	0.49
Mo sire: F2 vs (F1+F3+F4)	0.44	0.03	0.02	0.58	0.11	0.41	0.09	0.71	0.90
Ho sire: F3 vs (F1+F2+F4)	0.50	0.07	0.21	0.01	0.40	0.18	0.13	0.33	0.48

[1]SCS = 3 + log2 (SCC/100,000)

[2]Milk fat globule size: d_{43} = Average diameter; d_{32} = Sauter (surface) diameter

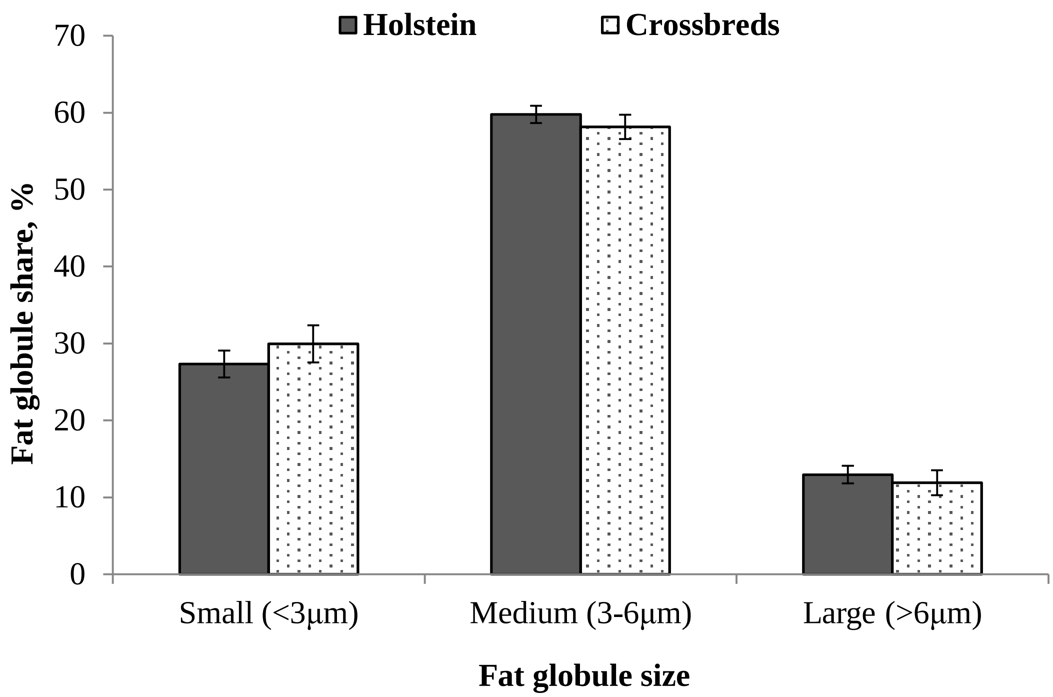

Figure 1. Share of fat globule size from milk samples of Holstein and crossbred cows (average of F1, F2, F3 and F4 breed combinations).

The least square means of single point MCP across different breed combinations are presented in Table 4. Milk from crossbred cows evidenced comparable coagulation time (RCT) with that from Holstein ones, but tended to have better curd firmness after 45 and 60 min since rennet addition and evidenced a shorter time to reach curd firmness 20 mm (k_{20}). In comparison among different crossbred cows, Mo sired crossbred cows had a tendency for higher coagulation time and lower curd firmness after 45 min than other sired (VR and Ho) crossbred cows.

Table 4. Least squares means and standard error of the means (SEM) for single point milk coagulation properties (MCP) across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Milk coagulation traits[1]				
	RCT, min	k_{20}, min	a_{30}, mm	a_{45}, mm	a_{60}, mm
Breed:					
Purebred Holstein (Ho)	25.0	6.5	26.6	34.1	37.0
VR×Ho (F1)	23.1	4.6	32.2	40.1	40.3
Mo×(VR×Ho) (F2)	26.8	6.4	27.4	33.9	38.4
Ho×[Mox(VR×Ho)] (F3)	23.8	5.9	29.8	36.7	37.0
VR×{Ho×[Mo×(VR×Ho)]} (F4)	25.4	5.7	24.6	38.6	40.9
SEM	2.2	0.7	4.3	2.7	1.9
Breed effect (*P v*alue)	0.35	0.15	0.61	0.22	0.20
Contrasts, *P* value:					
Ho vs crossbreds (F1+F2+F3+F4)	0.83	0.10	0.57	0.10	0.10
VR sire: (F1+F4) vs (F2+F3)	0.50	0.15	0.96	0.12	0.08
Mo sire: F2 vs (F1+F3+F4)	0.09	0.17	0.75	0.10	0.58
Ho sire: F3 vs (F1+F2+F4)	0.41	0.68	0.70	0.76	0.11

[1]RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} (a_{45}, a_{60}) = curd firmness after 30 (45, 60) min from rennet addition.

Table 5 reported the least square means for curd firming equation parameters across breed combinations. Moving to the curd firming equation parameters, estimated RCT_{eq} was comparable for crossbred cows to Holstein milk, although samples from crossbred cows showed greater curd firming (+ 16%, k_{CF}) and syneresis rate (+ 21%, k_{SR}) with improved maximum curd firmness (+ 8%, CF_{max}) and curd firmness potential (+ 8%, CF_p) compared to Holstein milk (Table 5; Figure 2) Compared within the crossbred cows, Mo sired crossbred cows displayed worsened curd firming equation parameters than VR and Ho sired crossbred cows (Table 5; Figure 3). On the other hand, VR sired crossbred cows showed better asymptotical potential and maximum curd firmness ($P < 0.05$, CF_p and CF_{max}) than Ho and Mo sired crossbreds.

Table 5. Least squares means and standard error of the means (SEM) for curd firming (CF_t) equation parameters across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	CF_t equation parameters[1]					
	RCT_{eq}, min	k_{CF}, % × min^{-1}	k_{SR}, % × min^{-1}	CF_p, mm	CF_{max}, mm	t_{max}, min
Breed:						
Purebred Holstein (Ho)	26.3	8.2	0.7	55.1	40.2	51.4
VR×Ho (F1)	23.6	10.4	1.0	62.5	45.6	46.4
Mo×(VR×Ho) (F2)	27.0	8.3	0.70	56.1	40.9	52.0
Ho×[Mox(VR×Ho)] (F3)	23.9	10.1	0.9	57.7	42.1	47.8
VR×{Ho×[Mo×(VR×Ho)]} (F4)	25.1	9.3	0.9	60.8	44.3	49.3
SEM	2.2	0.8	0.1	2.6	1.9	2.8
Breed effect (*P* value)	0.25	0.02	0.15	0.07	0.07	0.17
Contrasts, *P* value:						
Ho vs crossbreds (F1+F2+F3+F4)	0.24	0.02	0.08	0.03	0.03	0.14
VR sire: (F1+F4) vs (F2+F3)	0.44	0.35	0.18	0.05	0.05	0.35
Mo sire: F2 vs (F1+F3+F4)	0.07	0.03	0.07	0.10	0.10	0.07
Ho sire: F3 vs (F1+F2+F4)	0.41	0.30	0.88	0.45	0.45	0.54

[1]RCT_{eq} = RCT estimated according to curd firm change over time modeling (CF_t); k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness; t_{max} = time to reach CF_{max}.

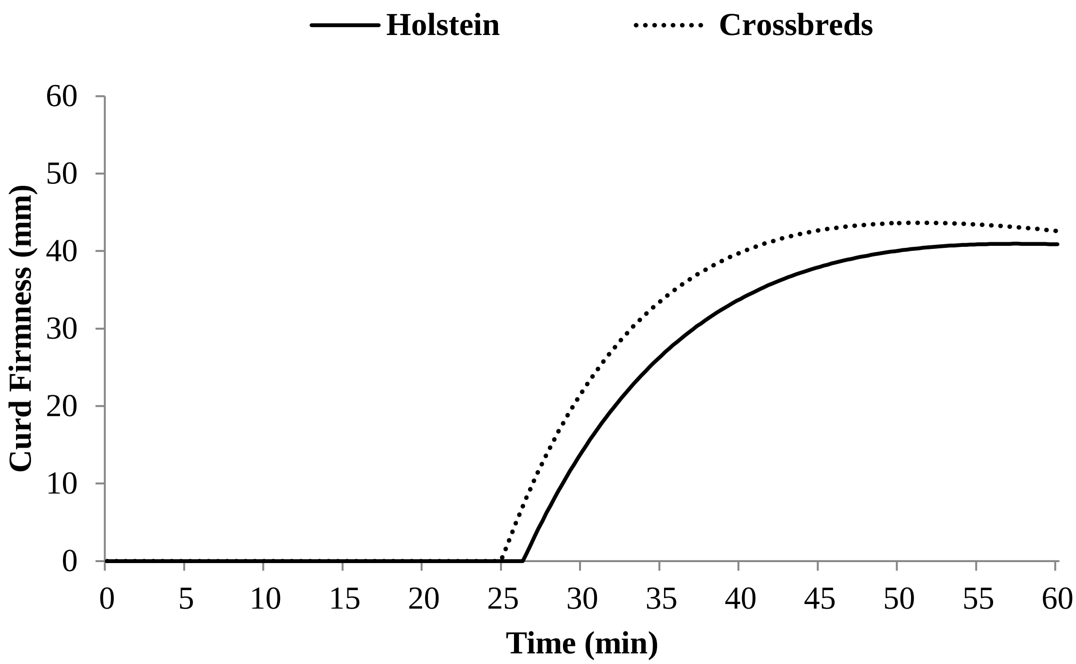

Figure 2. Pattern of curd firming after rennet addition (CF_t equation) of milk samples from Holstein and crossbred cows (average of F1, F2, F3 and F4 breed combinations).

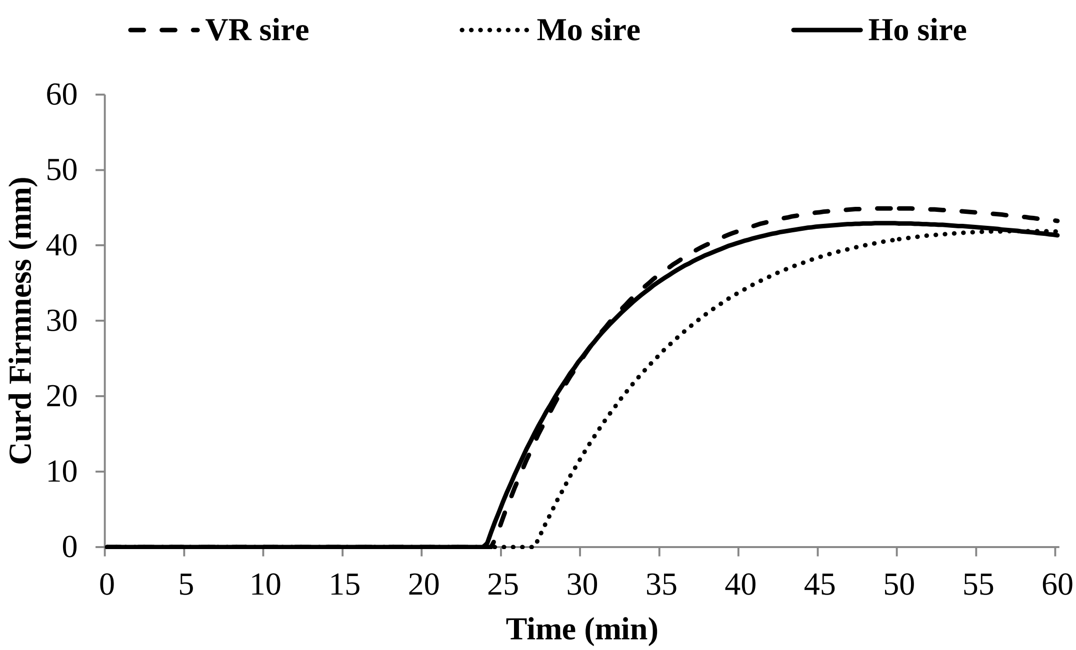

Figure 3. Pattern of curd firming after rennet addition of milk samples from VR (Viking Red) sire, Mo (Montbeliarde) sire and Ho (Holstein) sired crossbred cows

Yield, Weight, Rheological, Chemical and Sensory Attributes of Cheese

Least square means across different breed combinations for cheese yield and milk nutrient recovery in curd are given in Table 6. On average, the milk yielded was nearly 178 g curd/kg (40% solids and 60% water) with a recovered milk protein and fat close to 78 and 80%, respectively. Breed had effect on total curd yield by due to different water entrapped in the curd. Milk protein recovered in the curd was also influenced by breed effect. However, no differences were observed in the curd yield and milk nutrient recoveries in curd between Ho and crossbred cows. In the case of crossbred cows, VR sired crossbred cows yielded favorable curd yield and nutrients recovery in curd compared to other sired (Mo and Ho) crossbred cows. On the contrary, Mo sired crossbred cows exhibited worsened curd yield and milk nutrient recoveries in curd among different sired crossbred cows.

Table 6. Least squares means and standard error of the means (SEM) for fresh curd and cheese yield (CY_{70D}) and milk nutrient recovery in fresh curd (REC) across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Fresh curd yield[1], %			REC[2], %				CY_{70D},[3]
	Total	Solids	Water	Protein	Fat	Solids	Energy	
Breed:								
Purebred Holstein (Ho)	17.47	7.03	10.47	77.43	79.77	50.04	63.92	10.31
VR×Ho (F1)	19.25	7.56	11.69	78.21	81.79	52.29	65.82	11.23
Mo×(VR×Ho) (F2)	16.81	6.79	10.03	76.41	77.62	48.77	62.06	9.77
Ho×[Mox(VR×Ho)] (F3)	17.60	6.97	10.62	77.53	79.75	49.42	62.88	10.32
VR×{Ho×[Mo×(VR×Ho)]} (F4)	18.22	7.39	10.82	78.19	79.16	51.40	64.77	10.74
SEM	0.62	0.27	0.45	0.43	1.34	0.96	1.01	0.34
Breed effect (*P* value)	0.03	0.25	0.02	0.03	0.35	0.10	0.14	0.05
Contrasts, *P* value:								
Ho vs crossbreds (F1+F2+F3+F4)	0.29	0.50	0.27	0.68	0.87	0.61	0.97	0.49
VR sire: (F1+F4) vs (F2+F3)	0.01	0.04	0.01	0.01	0.23	0.01	0.01	0.01
Mo sire: F2 vs (F1+F3+F4)	0.01	0.09	0.01	0.002	0.11	0.04	0.05	0.01
Ho sire: F3 vs (F1+F2+F4)	0.45	0.01	0.57	0.88	0.90	0.22	0.30	0.52

[1]Total = total fresh curd yield; SOLIDS = total solids curd yield; WATER = water entrapped in the curd.
[2]$REC_{PROTEIN}$ = milk protein retained in the curd; REC_{FAT} = milk fat retained in the curd; REC_{SOLIDS} = milk total solids retained in the curd; REC_{ENERGY} = milk energy retained in the curd.
[3]CY_{70D} = cheese yield after 70 d of ripening

Least square means and orthogonal contrasts for cheese weight, weight loss, chemical composition and pH of cheese are reported in Table 7. On average, individual milk samples, close to1.5 L, yielded nearly 268 g of fresh curd post brine, and 158 g after 70 days of ripening; means ripening loss was then about 41.0%. Milk and cheese weights were very similar in Ho and crossbred cows. However, the fresh curd and cheese weights were differ within the crossbred cows, where VR sired crossbred cows yielded greater curd and cheese weight (8% and 9% respectively) than other crossbred cows. Conversely, Mo sired crossbred cows yielded lower curd and cheese weights. The average composition of prepared cheese was 69% DM, 46% fat and 40% protein in DM and the pH 5.12. The chemical composition of the cheeses made from Ho and crossbred cow's milk was very similar. Furthermore, there were no differences in cheese composition among crossbred cows.

Table 7. Least square means and standard error of the means (SEM) for milk weight, cheese weight (fresh and after 70 d), weight loss (after 70 d ripening), chemical composition and pH of cheese across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Milk weight, g	Weight		Ripening loss, %	Chemical composition (% DM)				pH
		Fresh curd, g	Cheese$_{70D}$, g		DM	Protein	Fat	Ash	
Breed:									
Purebred Holstein (Ho)	1519	265	157	40.85	68.4	39.4	46.4	5.94	5.09
VR×Ho (F1)	1518	292	170	41.58	69.1	38.9	47.2	5.71	5.05
Mo×(VR×Ho) (F2)	1520	255	148	41.68	68.3	40.6	45.1	6.11	5.11
Ho×[Mox(VR×Ho)] (F3)	1521	268	157	41.20	68.9	39.7	45.8	6.00	5.09
VR×{Ho×[Mo×(VR×Ho)]} (F4)	1521	277	163	41.25	68.0	40.7	45.0	6.00	5.07
SEM	2.89	9.23	5.06	0.72	0.64	0.92	1.13	0.17	0.07
Breed effect (*P* value)	0.86	0.03	0.05	0.80	0.62	0.52	0.61	0.58	0.85
Contrasts, *P* value:									
Ho vs crossbreds (F1+F2+F3+F4)	0.70	0.28	0.47	0.28	0.77	0.41	0.98	0.92	0.73
VR sire: (F1+F4) vs (F2+F3)	0.65	0.01	0.01	0.98	0.99	0.69	0.59	0.28	0.33
Mo sire: F2 vs (F1+F3+F4)	0.87	0.01	0.01	0.64	0.55	0.41	0.48	0.29	0.37
Ho sire: F3 vs (F1+F2+F4)	0.70	0.46	0.53	0.68	0.55	0.75	0.98	0.78	0.75

Table 8 shows the rheological properties of cheese across breed combinations. Rheological properties of cheese such as color and texture were measured by instrumental system. The color parameters were lightness (L*), redness (a*), and yellowness (b*). The mean values obtained for L*, a* and b* were 81.5, -0.8 and 14.4, respectively. The textural attributes of cheese refers to hardness, cohesiveness, elasticity and chewiness. In general, hardness means the maximum force needed to attain a given product deformation; cohesiveness indicated the strength of the internal bonds to make a body of the product; elasticity is the ability of the deformed product to resume its normal condition after the compression force remove; chewiness indicated the energy required to melt product for swallowing (Lakhani et al., 1991). The mean values obtained for texture attributes were 25.38 N, 072, 0.83 and 15.23 N for hardness, cohesiveness, elasticity index and chewiness, respectively. The cheese manufactured from the milk of Holstein and crossbred cows showed similar rheological properties (color and texture attributes). Also, rheological properties were same among crossbred cows except elasticity index for Ho sired crossbred cows.

Table 8. Least squares means and standard error of the means (SEM) for rheological properties of cheese across breed combinations (Ho = Holstein; VR = Viking Red; Mo = Montbéliarde)

	Cheese color[1]			Textural characteristics			
	L*	a*	b*	Hardness, N	Cohesiveness	Elasticity index	Chewiness, N
Breed:							
Purebred Holstein (Ho)	81.8	-0.79	14.5	24.15	0.72	0.83	14.45
VR×Ho (F1)	82.2	-0.69	13.9	26.28	0.72	0.82	15.23
Mo×(VR×Ho) (F2)	81.4	-0.75	15.0	24.53	0.74	0.82	14.98
Ho×[Mox(VR×Ho)] (F3)	81.1	-1.02	14.4	25.23	0.71	0.86	15.66
VR×{Ho×[Mo×(VR×Ho)]} (F4)	81.2	-0.77	14.4	25.07	0.72	0.82	14.15
SEM	1.07	0.16	0.37	2.84	0.02	0.01	1.85
Breed effect (*P* value)	0.65	0.51	0.36	0.92	0.54	0.27	0.93
Contrasts, *P* value:							
Ho vs crossbreds (F1+F2+F3+F4)	0.50	0.85	0.96	0.48	0.55	0.99	0.65
VR sire: (F1+F4) vs (F2+F3)	0.49	0.28	0.15	0.69	0.64	0.14	0.68
Mo sire: F2 vs (F1+F3+F4)	0.91	0.62	0.07	0.64	0.13	0.59	0.98
Ho sire: F3 vs (F1+F2+F4)	0.46	0.08	1.00	0.98	0.35	0.02	0.60

[1]L* = lightness, a* = redness/greenness, b* = yellowness/blueness

Table 9 shows the Pearson product moment correlations among sensory traits. Data indicated that correlation among sensory traits ranged between -0.41 to 0.51. Most of the sensory traits were significantly (positive or negative) correlated. Among 3 texture descriptors (elasticity, firmness and moisture), only elasticity was positively correlated with moisture (0.33), whereas firmness was negatively correlated with elasticity (-0.26) and moisture (-0.29). The association between smell and flavor intensities with color was positive. Moving to 5 taste descriptors (sweet, salt, sour, bitter and umami), sweet taste had a negative correlation with all other taste descriptors; whereas salt attribute had a positive association with sour (0.51) and umami (0.25) and sour attribute was positively correlated with bitter and umami (0.13, 0.21 respectively).

Color was positively correlated only with texture attributes of firmness. Also, color showed low positive correlation with smell (0.20) and flavor (0.19), and taste descriptors (salt and umami). We found that the Pearson correlations in terms of texture descriptors and smell were positive, whereas negatively associated with flavor. In most of the cases texture descriptors, and taste descriptors had either positive or negative association.

Table 9. Pearson product-moment correlations among sensory traits

	Elasticity	Firmness	Moisture	Smell	Flavor	Sweet	Salt	Sour	Bitter	Umami
Color	-0.03	0.20**	-0.07	0.20**	0.19**	0.02	0.17**	-0.03	-0.16**	0.23**
Elasticity		-0.26**	0.33**	0.10**	-0.05	-0.02	-0.41**	-0.23**	-0.08*	-0.10**
Firmness			-0.29**	-0.12**	0.01	0.10**	0.28**	0.12**	-0.11**	0.08*
Moisture				0.12**	0.04	-0.15**	-0.15**	0.01	0.14**	-0.10**
Smell					0.45**	-0.04	0.21**	0.06	0.04	0.22**
Flavor						-0.02	0.36**	0.24**	0.11**	0.32**
Sweet							-0.20**	-0.38**	-0.41**	-0.12**
Salt								0.51**	-0.001	0.25**
Sour									0.13**	0.21**
Bitter										0.04

*$P < 0.05$; **$P < 0.01$

In Figure 4, the four random effects of hierarchical linear model used for statistical analysis are illustrated: 1) sampling date, 2) animal within cow breed and parity class and lactation class, 3) sensory panelist and 4) the residual for sensory traits. As clearly seen in the Figure 4, residuals had the largest variability among all sensory traits except salt. The panelist mostly showed the second largest variability for sensory traits (magnitude of the variability was different). The effect of animal was much smaller than the residual and panelist variance, except for bitter and sour taste. The source of variation for sampling date was the smallest in all the random factors presented in the model.

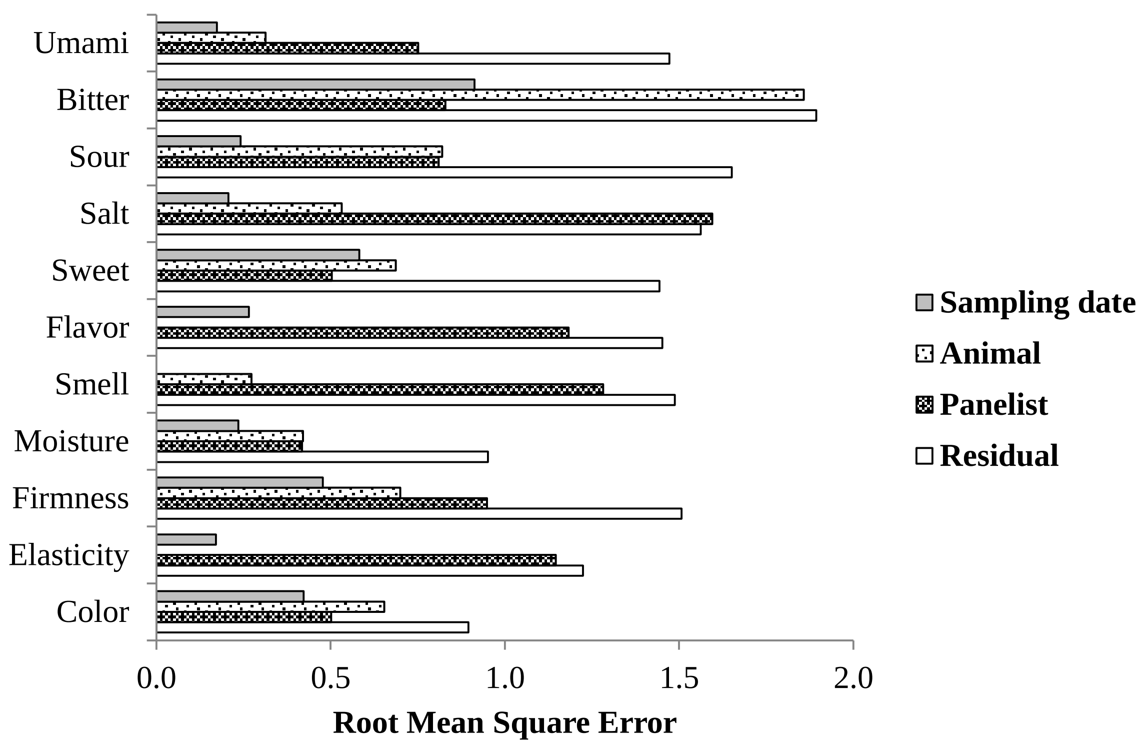

Figure 4. Root mean square errors of the date of sampling, animals, and panelist effects and of the residual of the sensory traits.

Regarding the sensory profile of cheese, all considered sensory descriptors were not affected by the breed effect, also the different sire effects on crossbred cows in this trial (Figure 5 and 6). The perception (score: low, 1 to high, 10) of panelist for taste descriptors revealed that cheeses made in this study were more sour (6.2), salty (4.7) and umami (5.2) in taste than bitter and sweet. Alongside, texture descriptors (elasticity, firmness and moisture) indicated that cheeses have been scored as more firm than elastic, probably because of the few moisture perceived. Moreover, the cheeses of this study tend to have intense flavor (6.1) and smell (5.6) with whitish in color. No differences have been found by panelists between the cheeses made with milk from Ho and crossbred cows (Figure 5). Moreover, cheeses made with the milk of crossbred cows sired by VR, Mo and Ho exhibited the similar sensory attributes (Figure 6). Cheeses made from the milk of Holstein and crossbred cows (Figure 5) or different sired (VR, Mo and Ho) crossbred cows (Figure 6) showed the same trend for sensory attributes in radar chart.

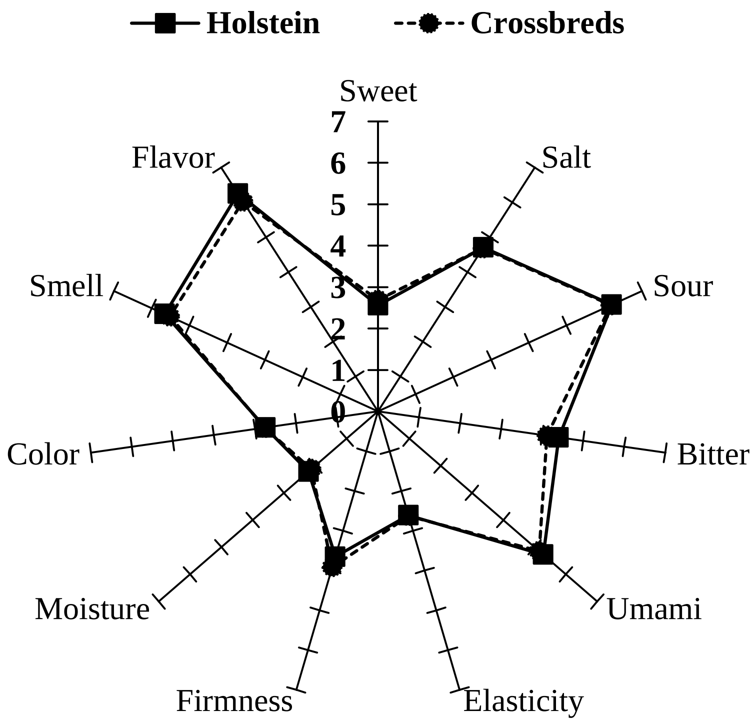

Figure 5. Least square means of sensory scores of cheese after 70 d of ripening from Holstein and crossbred cows (average of F1, F2, F3 and F4 breed combinations). Descriptors were evaluated on a 10 point set up scale from 1 (low) to 10 (high). Sensory score for all descriptors were not significance difference ($P < 0.05$) between Holstein and crossbred cows.

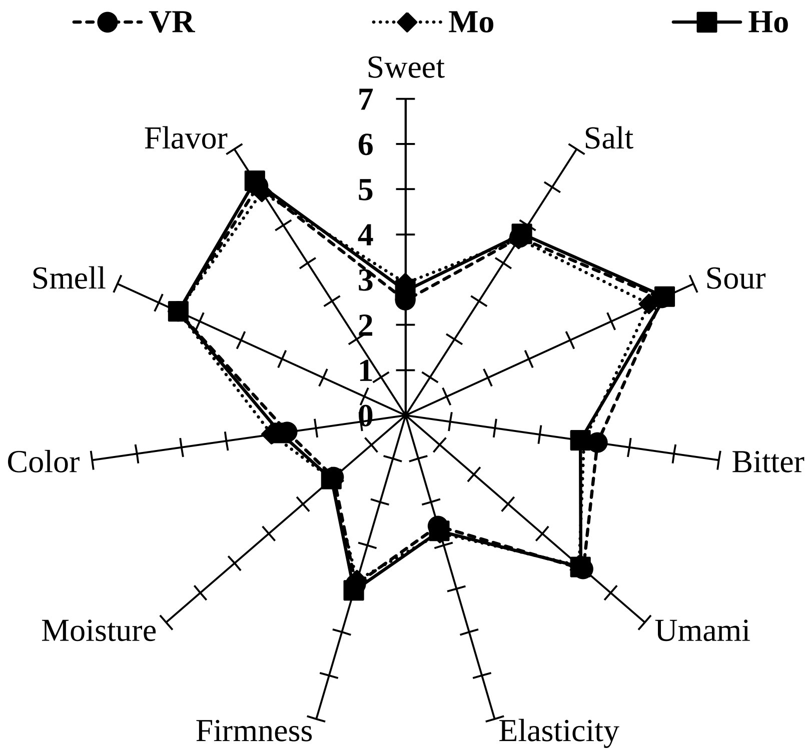

Figure 6. Least square means of sensory scores of cheese after 70 d of ripening from VR (Viking Red) sire, Mo (Montbeliarde) sire and Ho (Holstein) sire crossbred cows. Descriptors were evaluated on a 10 point set up scale from 1 (low) to 10 (high). Sensory score for all descriptors were not significant difference (*P* < 0.05) among VR, Mo and Ho sired crossbred cows.

DISCUSSION

Several studies on crossbreeding of dairy cattle have reported a positive effect of crossbreeding on functional traits (fertility, health, calving ease and longevity) of dairy cattle. In our previous study, milk production and milk quality, coagulation and cheese yield of crossbred cows has been studied using a 9-milca method. The present study has been designed to mainly focus on cheese quality traits of pure Ho and crossbred cows and also to evaluate eventual differences among crossbred cows using an individual model cheese-making method.

Milk Composition, Coagulation, Curd Firming and Curd Yield Traits

Our results suggest that crossbred cows were better for milk composition (protein and casein), SCS and casein micelle size than pure Ho cows. Our findings confirmed previous results by Hazel et al. (2017b) who found that crossbred cows (VR×Ho) had greater milk protein (+ 5%) than purebred Ho. Also Ezra et al. (2016) reported that Norwegian Red × Ho crossbred cows, which share the same genetic line with VR×Ho crossbreds, showed greater protein (+ 5%) percentage, but same fat content in milk compared to purebred Ho in their first lactation. Similar results have also been reported for three breed crosses using Ho, Mo, and VR sires on Ho cows (Malchiodi et al., 2014b; Shonka-Martin et al., 2019). Moving to different sired crossbred cows, found that VR sired crossbred groups (F1 and F4) had significantly higher protein and casein percentage than Mo (- 6%) and Ho (- 6%) sired crossbred cows. These results are in agreement with previous report that VR breed was superior to Ho cows for protein percentage in milk (Jönsson, 2015). Conversely, Mo sired crossbreed cows had lower protein and casein content in milk than other sired crossbred cows, and results are differed from other studies of Mo breed and Mo×Ho compared with pure Ho (Martin et al., 2009; Hazel et al., 2017b), perhaps the reason for different breed/genetic portion present in maternal line of crossbred cows.

Our findings that crossbred cows (2.68) had lower SCS than pure Ho cows (3.30) corroborate the previous study comparing Mo×Ho crossbred cows with pure Ho (Dezetter et al., 2015). Similarly, Heins and Hansen (2012) found lower SCS in Mo×Ho and Scandinavian Red×Ho crossbred cows compared to purebred Ho. In general, udder health, as indicated by SCS and relate with mastitis; a study by Clasen et al. (2019) reported that crossbred cows (Nordic Red×Ho) had lower incidence of mastitis than Ho when they were reared in commercial dairy farm with a high production level. Moving to different sired crossbred cows, VR sired crossbred

(F1 and F4) tended to lower SCS compared to Mo and Ho sired crossbreds, similar to the findings of Malchiodi et al (2014b) where Swedish Red sired crossbred had lower SCS compared to Mo and Brown Swiss sired crossbreds. Conversely, Swalve (2007) found VR×Ho had higher SCS than pure Ho in organic farming system.

Casein micelles are polydisperse, colloidal, roughly spherical particles and their size and structures are crucial for gel formation in cheese making (Glantz et al., 2010). The average casein micelle size of this study was comparable to the results from native Swedish dairy and Swedish Red cows (Poulsen et al., 2017). Crossbred cows had lower casein micelle size in milk compared to pure Ho. A study by Glantz et al. (2010) reported that smaller casein micelles were associated with better coagulation properties of milk. In addition, other factors like protein and calcium content and ionic calcium concentration of milk have also related to milk coagulation properties Gustavsson et al. (2014).

Milk fat globules consists of triglyceride, phospholipid and protein and their size range from 200 nm to over 15µm which can influence the physicochemical and sensory properties of dairy products such as cheese (Agrov-Argaman, 2019). The least square means for both traits (d_{43} and d_{32}) were comparable to the results from Ho cows (Couvreur et al, 2007). Several studies in the last decade have reported that species, breed, parity, days in milk, season, herd, diet and milking period can influence the milk fat globule size (Carroll et al., 2006; Couvreur et al, 2007; Ménard et al., 2010; Logan et al., 2014). However, to the best of our knowledge no previous studies have investigated the size of fat globule from the milk of crossbred cows, it was difficult to compare the obtained results.are difficult to compare.

Regarding milk coagulation properties, average MCP traits were comparable to our previous study, although the storing condition of milk samples was different, refrigerated in present study and frozen milk in the previous study. Crossbred cows had positive effect on curd firming parameters, such as lower time to reach curd firmness of 20 mm (k_{20}) and the greater curd firmness attained after 45 and 60 min of rennet addition. As reported in other study by Malchiodi et al. (2014b), time to reach curd firmness of 20 mm (k_{20}) and curd firmness after 30 min from rennet addition (a_{30}) were better for crossbred cows than Ho, even if Brown Swiss used as sire with Swedish Red and Mo. Variations in the MCP traits between crossbreds and Ho might be due to different breeds, milk composition and possible heterosis effects. MCP traits can be affected by breed (Bittante et al., 2012). Milk from Swedish Red cows (same genetic line of Viking Red) yielded slightly longer RCT than Danish Holstein with the same curd firming rate (Poulsen et al., 2013). Mo breed evidenced better milk coagulation than Ho cows (Martin et al., 2009; Bittante et al., 2012). Inside the breed, genetic variant of protein composition (Bittante et al., 2012) and protein content of milk (Poulsen et al., 2013) need to be considered for differences in MCP traits. Cows with higher k-casein and protein content in milk are responsible for better milk coagulation properties (Bittante et al., 2012; Poulsen et al., 2013). On this regard, milk from crossbred cows (sired by Swedish Red and Mo bulls) yielded higher casein and k-casein compared to Ho cows (Maurmayr et al., 2018) and the improved protein and casein content noticed in crossbred cows milk in this study could be a probable reason for favorable curd firmness properties of milk from crossbred cows. Lastly, we know heterosis has a positive effect for yield traits, but may have negligible effects on nutrient contents (Dezetter et al., 2015). Also, the literatures on effects of heterosis on MCP traits are not available and the data are not suitable for estimates the heterosis due to lack of pure VR and Mo information. Among different sired crossbred cows, MCP traits were similar with some sporadic differences. Also, Malchiodi et al. (2014b) reported some sporadic differences in MCP traits among different sired crossbred cows.

No available literature to compare the crossbreeding effects on curd firming traits of milk. Only a study conducted by Malchiodi et al (2014b) reported that milk from crossbred cows (sired by Brown Swiss, Swedish Red and Mo) showed favorable curd firming parameters than Ho cows and agrees with the findings of present study. In moving to different sired crossbred cows, Mo sired crossbred cows showed worsening curd firming parameters than other sired (VR and Mo) crossbreds.

Yield, Weight, Rheological, Chemical and Sensory Attributes of Cheese

Curd yield, nutritional characteristics, and sensory properties are mostly influenced by several genetic, environment and technological factors (Coulon et al., 2004). Animal genetic (breed) is one of the most important factor affecting the milk quality, coagulation and cheese yield properties and, consequently affecting quality and characteristics of the cheese (De Marchi et al., 2008; Bland et al., 2015). In the present study, average curd yield was nearly 2% greater than that of other studies dealing with individual cheese making (Cipolat-Gotet et al., 2013; Stocco et al., 2018) due to higher retention of water in the curd. As a result, ripened cheese yield (after 70 days ripened) was higher (+ 2%) than the findings of previous study by Cipolat-Gotet et al. (2018). The traits related to recovery of milk nutrient (fat, protein, solids and energy) in curd were almost similar to previous authors (Stocco et al., 2018; Cipolat-Gotet et al., 2013) mentioned reports.

A study by Stocco et al. (2018) reported that cheese yield traits were affected by breed of animal. Milk from Italian Simmental (same genetic group of Montébliarde) had greater cheese yield ability than Ho cows. Similarly, Martin et al. (2009) found that Mo breed yielded higher curd compared to Ho, whereas Verdier-Metz (1998) did not find any difference between these two breeds. Literatures on effect of crossbreeding or the role of heterosis on CY traits are not available to make direct comparison with our study. Only the findings of our previous study reported that milk from ProCROSS crossbred cows had comparable cheese yield and recovery traits with milk from Ho cows. In our previous study milk was stored in frozen condition and thawed before processing of milk might led to loss of fat which was exhibited in less recovery of milk fat in curd. Regarding different sired crossbred cows, VR sired crossbred cows had positive effect on curd yield and nutrient recovery traits (except fat recovery in curd) than other sired crossbreds, whereas Mo sired displayed opposite trend. A major explanation for this is the differences in water entrapped capacity in curd.

The weights of fresh curd and 70-d ripened cheese were similar for Holstein and crossbred cows. The cheese obtained with the milk from the Ho and crossbred cows were similar for rheological and sensory attributes. Moreover, no differences were observed in cheeses made from the milk of different sired crossbred cows. Only few studies have investigated the effects of breed of cows on cheese composition and sensory traits. Namely, Martin et al. (2009) reported that cheese made from Montbéliarde milk had higher fat and protein content and slightly lower pH and dry matter than Ho cows, whereas Verdier-Metz et al. (2000) did not find any differences in the quality of Saint-Nectaire type cheese among Ho, Montbéliarde and Tarentaise cattle breed. In addition, no differences were found in cheese composition between Friesian and Jersey cows (Auldist et al., 2004; Bland et al., 2015). Moving to color aspects of cheese, Martin et al. (2009) found cheeses from Ho and Mo cows displayed same lightness, red and yellow index. Conversely, Verdier-Metz et al. (1998) reported small differences in color of ripened cheese produced by Ho, Mo and Tarentaise cattle breeds. Texture was monitored both instrumental and sensory scoring process in our study. For instrument based textural measures, no texture differences were observed in cheese made from Mo and Ho cows (Martin et al., 2009). From the sensory standpoint, the cheeses made from the milk from Mo and Ho cows are somehow different in texture, taste and in aroma traits (Verdier-Metz et al., 1998; Martin et al., 2009). It is known that texture properties of cheese are influenced by moisture content and extent of primary proteolysis of cheese (Guinee, 2003; O'Mahony et al., 2005). Regarding the sensory profile, cheeses made from this study were slightly acidic, salty and umami in taste, which may have been due to the action of the native microflora present in milk, whereas no selective microbial strains were used in our cheese making process. Fox et al. (1990) reported that acidity increased in cheese probably due to the growth of lactic acid bacteria. Our prepared cheeses were also firm and low elastic to the touch. Indeed, low moisture content of cheese led to firm and less clastic cheese (Bland et al., 2015). Martin et al. (2009) reported that less elastic cheese were in salty taste compared to more elastic cheese. Furthermore, cheeses were made from this study tend to intense in flavor and smell, corroborate the findings of Papetti and Carelli (2013), who reported that native microflora's initially present in milk responsible for flavor production in cheese. Due to the lack of published data on rheological and sensory traits of cheese made from different breed combinations or crossbred cows, thus direct comparisons on those traits were fairly complicated.

In our study, correlation analysis among sensory traits provided additional insights relation/variation between observed sensory traits in cheese. Moisture had a positive correlation with elasticity, whereas negative correlation exhibited with firmness, as expected. Papetti and Carelli, (2013) reported that moisture content of cheese relate with hardness and the cheese became harder with an advancement of cheese storage period. A strong positive correlation was observed between smell and flavor intensities of cheese, similar to the findings of Cipolat-Gotet et al. (2018) in a study of sensory profile of model cheeses from Brown Swiss cows. Smell positively correlated with only two taste descriptors (salt and umami), whereas flavor correlated with almost all taste descriptors. Generally, perception of flavor attributes associate with taste descriptors (Small and Prescott, 2005). Sweet taste was negatively correlated with all other taste descriptors (salt, sour, bitter and umami) as expected. Reed et al. (2006) reported that Sweet is opposite to other taste descriptors because it is always perceived as a good taste. A strong positive correlation observed between salt and umami taste descriptors because sodium salt of l-glutamate produce umami taste (Hartley et al., 2019), whereas sour had weak positive correlation with bitter and umami taste descriptors.

CONCLUSIONS

This study was designed to investigate the effects 3 breed rotational crossbreeding system from milk to cheese level (whole feature of individual model cheese making), particularly giving emphasis on milk quality, coagulation, cheese yield ability and rheological and sensory properties of cheese, considering the performance of crossbred cows until fourth generation. Based on the results of this study, we conclude that compared to purebred Ho, crossbred cows produced higher content of protein and casein in milk with lower lactose content and SCS. Milk from crossbred cows had smaller casein micelle size than Ho cows, and this may contribute to improve the milk coagulation process. Milk coagulation and curd firming properties were slightly more favorable in crossbred cows compared to purebred Ho, without significant effects on the cheese yield and the recovery of milk nutrient in the curd. In addition, the weight, chemical composition, color, texture and sensory properties of cheese were comparable for crossbred and Ho cows. Thus, the result of this study confirmed that the use of 3-breed rotational crossbreeding system based on VR, Mo and Ho sire can be chosen even in the farm focused on PDO cheese production. Furthermore, VR sired crossbred cows had positive trend on milk composition (protein and casein), curd firming properties, cheese yield, milk nutrient recovery in curd and weight of cheese for fresh and ripened condition, whereas Mo sired crossbred cows had opposite trends.

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GENERAL CONCLUSIONS

This study investigated the variation in cheese making properties due to rumen acidity, summer transhumance of dairy cows and crossbreeding in dairy cattle. The results showed that variation in rumen acidity was associated with changes in the cow's rumination time and volatile fatty acids contents and composition of the rumen fluid. Milk yield was linearly decreased with increased rumen acidity whereas the milk composition, coagulation properties, cheese yield and nutrient recoveries in curd were not affected.

Moving to the effect of summer transhumance dairying system, the results showed that transhumant cows had lower body condition scores, reduced milk yield and milk protein content which confirms that transhumance system can affect productive functions and body condition of animal whereas the cheese making traits such as milk coagulation, cheese yield and nutrient recoveries in curd were not affected.

Crossbreeding in dairy cattle has recently become of increased interest. This study adds new knowledge about the long-term effects of a 3-breed rotational crossbreeding scheme on milk technological, cheese making properties and cheese characteristics, taking into account the performance of crossbred cows until the fourth generation of the rotational crossbreeding scheme. Results of this study confirmed that crossbred cows yielded less volume of milk compared to purebred Ho, but improved its content of protein and casein and lowered the somatic cell score. Also, milk from crossbred cows had smaller casein micelle size compared to Ho cows, and also fat globule size tended to be lower in the milk from CR cows. Milk from crossbred cows showed also better milk coagulation and curd firming properties than that of purebred Ho, without significant differences in cheese yield and milk nutrient recovery in the curd. In addition, the weight, chemical composition, rheological (color, hardness, cohesiveness, elasticity index and chewiness) and sensory attributes (color, smell, flavor, sweet, salt, sour, bitter, umami, elasticity, firmness and moisture) of produced model cheese were identical for Ho and crossbred cows. Therefore, the use of the 3-way rotational breeding system based on VR, Mo and Ho sires can be chosen even in farming systems specialized in cheese production. Future research is needed for studying interactions between crossbreeding schemes and heterosis effects of cheese making properties.

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LIST OF PUBLICATIONS

Manuscript under review

2019 **Saha, S**., N. Amalfitano, G. Bittante, and L. Gallo. Milk coagulation traits and cheese yield of purebred Holsteins and four generations of three-breed rotational crossbred cows from Viking Red, Montbéliarde, and Holstein bulls. *J. Dairy Sci.* (in review)

Publications in international Journals

2019 **Saha**, **S**., N. Amalfitano, E. Sturaro, S, Schiavon, F. Tagliapietra, G. Bittante, I. Carafa, E. Franciosi, and L. Gallo. Effects of summer transhumance of dairy cows to alpine pastures on body condition, milk yield and composition, and cheese making efficiency. *Animals*, 9:192.

2019 **Saha**, S., L. Gallo, G. Bittante, S. Schiavon, M. Bergamaschi, M. Gianesella, and E. Fiore. A study on the effects of rumen acidity on rumination time and yield, composition, and technological properties of milk from early lactating Holstein cows. *Animals*, 9:66.

2018 **Saha**, **S**., L. Carraro, G. Bittante, and L. Gallo. Body and milk quality traits of purebred Holstein and three-generation crossbred cows from Viking Red, Montbéliarde and Holstein sires. *J. Cent. Eur. Agric.* 19: 760–765.

2017 **Saha S**., F. Malchiodi, C. Cipolat-Gotet, G. Bittante, and L. Gallo. 2017. Effects of crossbreeding of Holsteins cows with Montbéliarde and Swedish Red in first and second generation on cheese yield traits. *Agric. Conspec. Sci*. 82:241–244.

ATTENDED CONFERENCES

Year	Name of the conferences
2019	70th Annual Meeting of the European Federation of Animal Science, Ghent, Belgium (with oral presentation)
2019	23° congress of the animal science and production association, Sorrento, Italy (with oral presentation)
2018	26th Animal Science Days, Pieštany, Slovakia (with oral presentation).
2018	69th Annual Meeting of the European Federation of Animal Science, Dubrovnik, Croatia (with oral presentation)
2018	1st European Symposium on Livestock Farming in Mountain Areas, Bolzano, Italy (with oral presentation)
2017	25th International Symposium Animal Science Days, Brandlucken, Styria, Austria (with poster presentation).
2017	22° congress of the animal science and production association, Perugia, Italy (with oral presentation)